CHEMORECEPTION STUDIES IN RELATION TO FEEDING RESPONSES IN THE MARINE SHRIMPS *PENAEUS INDICUS* H. MILNE EDWARDS AND *METAPENAEUS DOBSONI* MIERS.

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Dedicated to
My
Parents and brothers

DECLARATION

I hereby declare that this thesis entitled **"Chemoreception studies in relation to feeding responses in the marine shrimps Penaeus indicus H.Mile Edwards and Metapenaeus dobsoni Miers"** has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

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NOVEMBER 1995.

CHERYL HYACINTH FERNANDEZ

CERTIFICATE

This is to certify that the thesis entitled **"Chemoreception studies in relation to feeding responses in the marine shrimps Penaeus indicus H.Milne Edwards and Metapenaeus dobsoni Miers"** is the bonafide record of the work carried out by Miss. Cheryl Hyacinth Fernandez under my guidance and supervision in CMFRI and that no part thereof has been presented for any other degree.

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CONTENTS

LIST OF PLATES

LIST OF FIGURES

PREFACE

Successful and sustainable aquaculture of finfishes and crustaceans depends upon the provision of nutritionally adequate, environment - friendly and economically viable artificial feeds. Feed is the major operational input, and feed cost normally range from 30 to 60 percent of the operational expenditure in finfish and prawn culture systems.

In extensive and semi-intensive culture systems, natural food contributes substantially to the nutrition of the cultured animals. The production from such systems depends on the natural biogenic potential, the quantity and quality of natural food produced in the pond and artificial feed supplied and on the physico-chemical characteristics of the water and soil. But in the intensive system of culture, where very high stocking densities are maintained, the cultured animal has to rely almost exclusively on the artificial feeds fed to them.

But despite all the recent technological development a reversal in the rapid increase of world shrimp production from 2.12 MMT of 1985 to 2.912 MMT of 1992, occurred in 1993 with a total of 2.71 MMT. This declining trend is being blamed on several factors; unstable market prices, high production costs, onset of diseases, weather and most important of all the degradation of the pond environemnt. Aquaculture has expanded and intensified to such a large extent that it can now be considered a potential polluter of the aquatic environment. As farms evolve from low densities to high densities there is almost no natural food production used. As stated this operation is dependent on using high quality feeds which offer the advantages of better feed conversion, faster growth and high survival.

But the high stocking rates and intensive feeding will increase the organic load of rearing water. Inorder to prevent loading of too much organic waste into aquaculture systems, it is important to use feeds that minimise the amount of nutrient leaching, should be readily acceptable and eaten quickly and have high degree of pellet integrity and water stability. But even from a well formulated, and stable feed, as much as 77% of the nitrogen and 86% of the phosphorous compounds in shrimp feed were wasted. So it is important that instead of increasing the pellet stability beyond a couple of hours, feeds should designed to be easily acceptable and palatable to the target organisms and should be consumed within the shortest time possible. Therefore, it is important to have effective feed attractants, protected vitamins and minerals.

Careful attention of the basic crustacean behavioural patterns is critical in order to provide an accurate system for evaluation of single and mixed chemical signals by the crustacean, resulting in more efficient utilization of the feed. The shrimp's feeding habits change from continuous to nocturnal as size increases. There are also shifts in preference for prey over the rearing period. These feeding rhythms and food preferences reinforce the need to manage the culture system as a total trophic environment. Different stages of the life cycle may require specific feed or prey types. Such information should ultimately lead to the development of innovative crustacean diets, geared specifically to the unique physiological stage of development.

A properly formulated diet is very important. The feed dependent factors are the most important among the factors affecting the efficiency of a feed and its cost. A proper diagnosis of these factors is of utmost importance in developing quality controlled least cost diets. Requirements will vary with different penaeid species and different ecological situation. Correct

formulation is critical to increase feed utilization and pond productivity, as well as the profitability of the operation. Growth rates in shrimp pond can be improved by decreasing feeding response time. A shortened response time means nutrients will have less opportunity to leach from the food pellet. In addition, less energy is expended by the shrimp in search of food.

Feed ingredients such as fish solubles, squid meal, mussel, flesh, shrimp meal, short necked clam, blood worms etc. contains a a variety of feeding stimulants ie. aminoacids, betaine, nucleotides and other elicit compounds which may also enhance the biological as well as the nutritional value of an aquatic diet. Extractives of a variety of invertebrate tissues have been demonstrated to contain attractive feeding properties for a variety of marine animals. Similar beneficial effects for mixtures of amino acids and nitrogenous bases have been documented.

Attractiveness and palatability are critical factors in formulation of aquatic diets, especially in weaning of young ones from natural foods to dry pelleted diets. The need for attractants and stimulants were best studied,

- By identification of feeding stimuli often needed to obtain the full sequence of feeding behaviour.
- response varies among aquatic species and is related to different chemosensory sites and thier interaction with a specific chemical or group of chemicals.
- understanding the feeding behaviour of the aquaculture species necessary in order to obtain an optimal feeding regime.
- rapid and positive uptake lessens residual time in the aqueous system.

- study the factors that influence feeding behaviour and response of cultured animals to a particular diet like water quality and nature of the test diet.

This present study involves the identification of the chemical signals that leads to feeding responses, the different sites of the animal body that are involved in chemoreception, effect of water quality parameters like pH and salinity on the shrimps feeding response, the effect of chemoattractants and stimulant on the feeding intake and growth of shrimps.

The thesis includes chapters like introduction, review of literature, materials and methods which includes the various methodologies used to identify chemical signals, results, discussion, summary, conclusion and references.

Introduction

INTRODUCTION

The most ideal crustacean candidates for commercial aquaculture are aquatic decapods such as shrimps, crabs and losters. In view of the tremendous growth in shrimp aquaculture in the recent years more and more attention is being given to the development of proper dietary formulations for the cultured organisms. Different combinations of animal and plant resources are being used for the manufacture of low cost diets. The cost of feed alone forms more than 50% of the production cost in shrimp aquaculture which also determines the profitability of the operation (Akiyama et.al., 1992). The aquaculture productivity can be improved through the use of proper feed and efficient feeding management. A high quality feed will improve growth, food conversion efficiency and subsequently reduce the production cost. This goal can be achieved by;

- Designing a proper feed to meet the nutritional requirements of different stages of growth;
- Improving the dietary delivery system,
- Tailoring micronutrients to the specific nutritional needs of individual species,
- Enhancing palatability to facilitate increased feed uptake,
- Using feed attractants and stimulants that promote feeding and
- Reducing the feed wastage.

Shrimp nutritionists and culturists have consequently developed formula diets that reduce feeding loss. The nutritional value of formula diets is normally evaluated by how the composition of essential nutrients meets the requirement of target organisms.

But feeding responses of the animals varies with the dietary acceptability and palatability associated with the physical and chemical properties of the diet, as well as the surrounding environmental conditions (Atema, 1980; Takeda, 1980a) and the nutritional status of the animals. In the aqueous medium some of the soluble nutrients may leach into the surrounding medium, making it unavailable for growth. An unstable feed which disintegrates in water in less than an hour can lose as much as 20% to 30% of its nutrients in the pond water and sedement (World Shrimp Farming, 1994). Moreover the shrimp's habit of slowly nibbling the feed causes substantial nutrient loss even if the pellets are of good quality. Within an hour shrimp feed will lose more than 20% of its crude protein; 50% of its carbohydrate, and 85 to 95% of its vitamin content. As feed alone constitutes bulk of the production cost; this nutrient loss represents a very significant cost factor. This unconsumed feed; in addition to increasing the production cost increases the level of metabolites in water, develop algal blooms and destroys substrate quality, through organic pollution.

An artificially compounded diet for these animals must be chemically attractive enough to enable location and elicit feeding within a short period of time (New, 1976). So instead of increasing the pellet stability beyond a couple of hours, feeds should be designed so as to be consumed within a short period of less than one hour. A shortened response time means soluble nutrients will have less opportunity to leach from the feed pellet (Deshimaru and Yone, 1978; Heinen, 1980). In addition less energy will be expended by shrimps in search of food.

So apart from nutritional value and pellet stability; food detection and feeding stimulation are significant factors in the design and formulation of aquatic diets. An otherwise nutritionally balanced diet may be less effective

or marginal in performance due to the absence or minimal concentration of ingredients that elicit positive stimulatory response in particular species. The efficiency of an aquaculture feed depends in large part on its being rapidly recognized as an acceptable food. Chemoattractant and stimulant that enhance the recognition are receiving considerable attention throughout the aquaculture feed industry (Gill, 1989; Ward, 1991).

In view of this importance of diet development in aquaculture more and more research is encouraged on dietary supplementation with feeding attractants and stimulants to improve the acceptability and palatability of the diets, especially for slow feeding crustaceans. The incorporation of feeding attractants and stimulants is a useful means to increase the palatability of formula diets for shrimps which in turn will result in an increase in satiation amounts and ultimately results in a reduction of feeding time.

A wide variety of aquatic animals are known to utilise chemical signals in search, location and ingestion of food. Attempts to identify and isolate active components of food extracts have been done by several workers for various species. All the same generalization can be made as to the chemical nature of feeding stimulants, ie•they are of low molecular weight, non volatile, nitrogenous and amphoteric compounds. A question still remains as to how and what chemical components; which shrimps use in food detection and ingestion. This uncertainty is partly due to differences in methodologies employed and partly due to simultaneous involvement of olfactory and gustatory systems. Some chemical stimulants may act as attractant via olfaction and others may act as promoters or enhancers of food intake or ingestion. Diet supplemented with attractants or stimulants stimulate feeding activity leading

to enhanced growth performance and survival. These effects attribute primarily to the improved food intake, digestion and absorption.

Although numerous studies on feeding attractants and stimulants for aquatic animals have been conducted in the recent, past and basic knowledge have been accumulated, there is little information on the possible application of this in aquaculture. Feeding stimulants are becoming increasingly important throughout the aquaculture feed industry to enhance animal response towards feed. Fish solubles, squid meal, mussel flesh, shrimp meal, various invertebrate extractives etc.as well as different synthetic chemicals like amino acids, nucleotides and sugars are variously included in different aquatic diets to impart specific attractive properties for the diet. Natural feed ingredients also contain a variety of feeding stimulants like amino acids, betaine and nucleotides, which may also enhance the biological as well as the nutritional value of aquatic diets.

Species often differ in their specificity towards different stimulants. So identification of the specific feeding stimuli for each species is needed to obtain the full sequence of feeding behaviour from initial recognition to feeding including ingestion of the food. These response varies among aquatic species and is related to different chemosensory sites and their interactions with a specific chemical stimuli. In addition to providing information on the chemoattractants and stimulants, basic information on chemosensory physiology and feeding behaviour also have significant practical application in formulation and commercial production of attractants and chemical baits in aquaculture as well as products used in sports and commercial fisheries.

When exposed to chemical stimuli related to feeding; shrimp initiate food search behaviour. Feeding behaviour is a stereotyped sequence of behavioural

components which can normally be differentiated into several phases such as perception, orientation, displacement, arrival and ingestion. Animal behaviour may vary depending upon the particular chemical signal and a single signal or combination of signals which may induce seperate responses. Proper understanding and careful characterization of feeding behaviour of each species is necessary to establish an optimum feeding regime for more efficient utilization of a particular feed with minimal expenditure of energy and resultant reduction in feed wastage and cost of feeding.

For the systematic study of feeding behaviour in decapods a reliable assay system is needed, which will enable proper monitoring and evaluation of animal's response to feed and feeding stimuli. As stated by Meyers (1986; 1987) such responses are of importance in aquaculture because types of feed and animal's feeding behaviour are both crucial for successful aquaculture.

Aquatic animals especially shrimps rely upon informations received through all the sensory channels. So proper understanding of the chemosensory system of the animals is also essential for the feeding, behavioural and chemotactic evaluation. The relative importance of individual sense organs differ in various species and is determined by their ecological niches, feeding strategy, motivation and other biotic and abiotic environmental factors. Chemoreceptors involved in distance chemoreception could be important for orientation towards source of food and food stimuli and those involved in contact chemoreception may play important role in food gathering and ingestion. Feeding responses have been used as a behavioural criteria for evaluating the chemosensory abilities of different chemoreceptors in crustacea.

Many studies have been made of the factors involved in stimulating feeding in crustaceans, particularly with lobsters, crabs and freshwater prawns. However little is currently known about chemoreception and the effect of attractants and stimulants on the feeding behaviour and ingestion in Penaeus indicus, one of the most widely cultivated penaeid shrimp along the Indian coast.

The aim of the present study is to identify and evaluate the potency of various potential feeding attractants and stimulants for P.indicus and Metapenaeus dobsoni by way of both behavioural and ongrowing studies. This approach allowed an assessment as to whether the behavioural responses to feeding stimuli can predict the effect of its supplementation on subsequent ongrowing performance. This measure is important as the addition of feeding attractant might elicit an increase in the appetite and subsequently food intake, assimilation and growth (Lindstedt, 1971) and also improve survival (Heinen, 1980).

Review of literature

REVIEW OF LITERATURE

1. CHEMORECEPTION IN FEEDING

Feeding is the major component of aquaculture management determining the productivity of the system and the cost of which alone alone plays a major role in the profitability of shrimp culture (Akiyama et.al., 1992). Commercial feeds are evaluated on a cost effective basis which weighs variables such as growth rate, food conversion efficiency and survival against the cost and availability of feed components (Urban and Pruder, 1991). The efficiency of a feed depends not on the nutritive value alone but also on it being recognized as an acceptable food by target organisms. For the formulation of an acceptable feed for aquaculture, the response of the target organisms to their prey and potential food are of importance (Meyers, 1986; 1987).

Aquatic crustaceans possess a well developed chemical sense to detect behaviourally relevant chemical cues from the background of "chemical noise" that must characterize aquatic environment (Kaissling, 1977). In crustaceans chemosensory stimulus is more important in food recognition, location and feeding than the visual stimulus (Bateson, 1889; Bell 1906; Symonds, 1964; Hazlett, 1968; 1971b; McLeese, 1970; Dahm, 1975). According to them visual stimulus plays only a secondary role or is not involved in eliciting feeding responses. The post larvae of Penaeus merguiensis (Hindley, 1975) and Macrobrachium rosenbergii (Moller, 1978) and sergestids and other penaeids (Benfield and Aldrich, 1992) use only chemotaxis coupled with rheotaxis in sensory perception of food and no visual cues are involved. In a behavioural study it was observed that zoea of the fiddler crab Uca pugilator (Herrnkind,

1968, Robertson et. al., 1981) and post larvae of M.rosenbergii (Moller, 1978) released the unsuitable food particles immediately after its capture, confirming the chemosensory discrimination in feeding. Chemoreception have functions other than feeding, such as location of hosts (Ache 1975), sexual attraction and recognition (Dunham 1978; Bauer, 1975) and detection of predators (Kittredge et. al., 1974).

Chemorception studies based on behavioural evaluation as early as 1894 established that dissolved substances are adequate stimuli for aquatic crustaceans and that chemosensitivity is concentrated on specific appendages (Nagel, 1894). Later studies by Case and Gwilliam (1961), Crisp (1967) and Laverack (1963) confirmed the above findings that external chemical agents are involved with diverse phenomena such as feeding behaviour, host location by commensals, mate recognition and prey concealment in crustaceans. Bardach and Villars (1974), Atema (1980) and Zimmer-Faust et. al., (1984) also observed that different chemosensory compounds are involved in each stage of feeding behaviour. These compounds play an important role in food detection and feeding especially in slow feeding crustaceans which reduces the loss of water soluble nutrients by leaching (Deshimaru and Yone, 1978; Heinen, 1980) and is receiving considerable attention in the aquaculture food industry (Gill, 1989; Ward, 1991). Lindstedt (1971) also observed that these compounds increase the appetite of the animal and subsequently increase in food intake, growth and survival.

Most of the studies on chemoreception in crustaceans were aimed to identify the potential feeding attractants and to analyse the effects of varying levels of such compounds on the feeding responses, particularly with regard to their effects on the chemosensory mechanism by behavioural and

electrophysiological means (Carr, 1978; Carr and Thompson, 1983; Carr et.al., 1984; Carr and Derby, 1986; Harpaz et.al., 1987; Harpaz and Steiner, 1987; 1990; Nakamura, 1987). The effects of such these selected compounds on the feed intake and growth of crustaceans have been further evaluated by several workers using both commercial (Farman Farmaianet.al., 1979; Murai et.al., 1981; El Hag, 1984; Costa-Pierce and Laws, 1985) and semi-purified diets (Deshimaru and Yone, 1978; Pascual, 1980; De Proenca, 1990; Hartati and Briggs, 1993).

2. BIOASSAY SYSTEMS

Different assay systems have been developed by various workers to evaluate the behavioural responses of animals in relation to feeding chemical stimulation. Mackie and Shelton (1972) designed a system consisting of a chamber with shelter habitat and one way mirrors for direct observation of the lobster's behaviour, when exposed to potential attractants, without the observer influencing their behaviour. McLeese (1972) developed a system in which the behavioural movements of the lobsters were traced using ultraviolet light after blinding and tagging them with fluorescent paint. Behaviour of the mature crab, Cancer magister to water from a pre-soaked bait were evaluated using in a large tank by Allen et.al., (1975). This system enabled direct behavioural observations. Bayer et.al., (1982) designed an apparatus to measure and record the direction of movement and speed of the lobsters in response to feed attractants with minimal observer influence, using photo electric cells. The system developed by Benfield and Aldrich (1991 and 1992) enables the measurement of the shrimps response towards olfactants for developing specific threshold levels of response.

A feeding bioassay system which uses agar discs was developed by Holland and Borski (1993) for evaluating chemosensory stimuli influencing ingestive behaviour in Penaeus vannamei. This system was found suitable for rapid screening of a wide variety of compounds. Steiner and Harpaz (1987) used videorecording to study the sequence of intake and rejection of pellets flavoured with different compounds.

3. CHEMORECETION STUDIES IN DECAPODS

Behavioural, physiological, and electrophysiological responses have been employed by several workers for evaluating chemoreception in decapods.

3.1. BEHAVIOURAL EVALUATION

Several investigators have described the feeding behaviour of marine (Fuzessery and Childress, 1975; Carr, 1978; Ache, 1982; Derby and Atema, 1982b; Devine and Atema, 1982; Johnson et.al., 1986b; Zimmer-Faust et.al., 1984; Atema, 1985; Carr and Derby 1986b; Zimmer-Faust, 1987) and freshwater crustaceans (Moller, 1978; Tierney and Dunham, 1982; Holland, 1985; Harpaz and Steiner, 1987; Harpaz et.al., 1987b; Steiner and Harpaz, 1987) towards actual food items or chemostimulants. Flicking of antennules, walking or swimming, grasping and lifting movements of pereopods and movement of mouth parts are the common behavioural indicators used in the chemoreception studies. Antennular flicking is considered as an arousal response (Ache et.al., 1976; Pearson and Olla, 1977; Pearson et.al., 1979; Holland, 1985) and swimming or walking movement as food search behaviour (Mackie and Shelton, 1972; Murai et.al., 1981; Carr et.al., 1984; Zimmer-Faust et.al., 1984; Harpaz et.al., 1987b) in crustaceans.

Costero and Meyers (1993) developed a series of behavioural descriptors such as perception, orientation, displacement, arrival and ingestion using chemoattractant coated feeds in P.vannamei to evaluate their feeding behaviour. Food seeking behaviour of P.merguiensis (Hindley, 1975), marine crabs Callinectes sapidus and Cancer magister (Pearson and Olla, 1977; 1979; Pearson et.al., 1979) and fresh water prawn M.rosenbergii (Harpaz et.al., 1987a; b, Harpaz and Steiner, 1987; 1990) have been described in detail.

3.1.1. **Arousal Behaviour:** Among the behavioural responses antennular flicking is considered as an arousal behaviour and is one of the most sensitive indicator of chemostimulant detection, used in the behavioural evaluation in crustaceans (Mackie and Shelton, 1972; Snow, 1973a; Fuzessery and Childress, 1975; Price and Ache, 1977; Pearson and Olla, 1977; Fuzessery, 1978; Harpaz et.al., 1987a). Since antennular flicking response is a wide spread behavioural manifestation among crustaceans, interspecies comparison is rather difficult because of variation in methods and diversity of chemostimulants used besides inherent differences between marine and freshwater species.

Flicking movements of antennae splays out the tightly packed aesthetascs (Chemoreceptive sensilla) presumably allowing increased exposure to surrounding chemical environment leading to temporal enhancement of the response of the primary receptors to weak odours (Snow, 1973a; Schmitt and Ache, 1979). Cinemetographic analysis in the hermit crab, Pagarus by Snow (1973a) indicated that flicking serves as a flushing mechanism facilitating water circulation around the sensillary hairs and consequently produces sampling of dissolved chemical in the crabs immediate environment. Similar observations were made by several workers in lobsters also (Price and Ache, 1977; Schmitt and Ache, 1979; Moore and Atema 1988). Price and Ache (1977)

analysed the effect of flicking on the discharge pattern of antennular chemoreceptors in the lobster Panulirus argus by physiological means. According to him during the interflick period water trapped between the aesthetasc form a boundary layer within which odourant movement takes place by molecular diffusion and the chemoreceptor response can be enhanced or depressed based on the antennular nature of chemical cues.

Fuzessery (1978) compared the functional significance of antennular flicking with the sniffing behaviour of terrestrial vertebrates.

Numerous environmental factors can alter the spontaneous frequency of flicking including the presence of natural chemical stimuli associated with feeding (Maynard and Dingle, 1963; Ai and Takei, 1973a; Snow, 1973a; Rainbow, 1974). Rate of this activity increased in the presence of chemical cues, implying that this behaviour serves to enhance reception (Fuzessery and Childress, 1975).

3.1.2. **Recognition/Food picking Behaviour:** In decapod crustaceans the chelate appendages are used for food recognition and picking (Derby and Atema, 1982a; Borroni et.al., 1985). Chela flexion is a common behaviour associated with food recognition among these animals. So it can be used as a standard measure to study chemical feeding stimulants and inhibitors in crustaceans (Johnson and Atema, 1986; Case and Gwilliam, 1961; Field, 1977). Compounds which stimulate food recognition and picking behaviour in other crustaceans are generally found to stimulate chela flexion in shrimps (Ache, 1982; Zimmer-Faust et.al., 1984; Carr and Derby, 1985) and is dependent both on the stimulus specificity and stimulus concentration (Trott and Robertson, 1984).

3.2. ELECTROPHYSIOLOGICAL EVALUATION

In order to identify and evaluate the chemoreceptor sites and to study their specificity to different stimuli electrophysiological studies have been conducted by several workers (Shepheard, 1974; Ache et.al., 1978; Derby and Harpaz, 1988). In this, impulses from nerve bundles of the chemoreceptor sites are recorded by exposing the receptors to the test stimuli. Response of M.rosenbergii to betaine-HCl was evaluated electrophysiologically by Derby and Harpaz (1988).

Fuzessery and Childress (1975) reported that electrophysiological responses and thresholds were several orders of magnitude higher than that of behavioural ones. It may be possibly due to technical recording difficulties or the existence of more sensitive receptors than the one monitored. Receptor sites also exhibited great variability in sensitivities and specificities (Ache, 1972; Shepheard, 1974; Tazaki and Shigenaga, 1974; Price and Ache, 1977; Fuzessery et.al., 1978b; Johnson and Ache, 1978). McLeesee (1970) also found little or no correlation between behaviour and electrophysiological responses of antennules of the lobster, Homarus.americanus. So the electrophysiological techniques appears to have limited value in chemoreception studies including screening of chemicals for behavioural studies.

3.3. PHYSIOLOGICAL EVALUATION

Physiological methods have been used by few workers to investigate the sensitivity of receptors and to evaluate chemical stimuli (Laverack, 1963; Case and Gwilliam, 1961; Case, 1964; Johnson and Ache, 1978). The only physiological response used so far to indicate chemoreception has been

alteration in heart rate induced by chemostimulants (Zimmer et.al., 1979). Physiological responses of the antennae and first walking leg of crabs, Carcinus maenas and Portunus sp. and lobster, Homarus gammarus showed that glutamic acid, glutamine and taurine are strong stimulants for this appendages (Laverack, 1963; Case and Gwilliam 1961; Johnson and Ache, 1978). However, the relation between the physiological response and behaviour in crustaceans have not been established.

4. FEEDING EFFECTORS

Invertebrates living in littoral habitats detect stimuli against chemical noise level in the back ground (Atema, 1985). Feeding behaviour and chemoreceptor responses of littoral crustaceans are stimulated by low molecular weight substances, such as animo acids and structurally related compounds (Ache, 1982), which are the secretions from the body of their prey or zooplanktons (Daumas, 1976; Davis et.al., 1985; Dawson and Goeke, 1978).

4.1. NATURAL STIMULI

4.1.1. **Tissue Extracts:** Several workers have studied the feeding attractant property of natural fluids or extracts of natural materials including the prey organism (Mackie, 1973; Takei, 1977; McLeese, 1970; Mackie and Shelton, 1972; Johnson and Ache, 1978; Zimmer-Faust and Michel, 1980). Natural extracts, such as those from squid (Mackie, 1973), crab (Carr et al., 1984) and other prey species (McLeese, 1973b) are more stimulatory in crustaceans than any other compounds.

A mixture of components of squid extract is more effective stimulant for lobster H.gammarus (Mackie and Shelton, 1972; Mackie 1973), 70% ethanol extract of squid skin in crab, Erimacrus insenbeckii (Takei, 1977), shrimp

extract in Panulirus argus (Johnson and Ache, 1978) and shrimp and lobster muscle extract in H.americanus (McLeese, 1970) than any other individual component. Whole prey extracts and complex mixtures were more effective than single compounds (McLeese 1970;) and a single class of compound was not as attractive as a complex mixture of several of these classes (Mackie and Shelton, 1972; Mackie, 1973;Zimmer-Faust and Michel, 1980).

4.1.2. **Other Natural Stimuli:** Many natural materials like flesh, meat solubles, human serum and oval albumin were found to evoke feeding behaviour in crustaceans by several workers (Liao, 1966; New, 1976; Sick, 1976; Pascual and Bandonil, 1977). Penaeus japonicus preferred the meat of short necked clam than squid meat and marine worm Pireinereis brevicirrus (New, 1976, Liao, 1966). Among the various soluble preparations Penaeus aztecus preferred clam solubles (Shewbart et.al., 1973) and among meal based diets prawn readily accepted shrimp head meal based pellets (Pascual and Bandonil, 1977).

Ovalbumin, and to a lesser extent ovaglobulin acts as an ingestant and when incorporated in the larval feed for M.rosenbergii increased the caloric ingestion rate (Sick and Beaty, 1975; Sick, 1976). Human serum was also found to act a feeding response inducer in Palaemonetes pugio (Carr and Gurin, 1975).

4.1.3. **Feeding Effector Component of Natural Stimuli:** Analysis of natural fluids and other natural materials attractive to crustaceans showed that major stimulants were substances of low molecular weight especially amino acids and related compounds (Case and Gwilliam, 1961; Mackie and Shelton, 1972; McLeesee, 1973b; Carr and Gurin, 1975; Ache et.al., 1976; Takei, 1977; Carr, 1978; Johnson and Ache, 1978), nucleotides (Carr et.al., 1984), organic bases,

guanidino compounds, organic acids and sugars (Konosu et.al., 1966). Carr and Gurin (1975) reported that substances of higher molecular weight (> C.1000 mw.) like proteins were primarily responsible for the attractiveness of the oyster mantle fluid, human serum and clam extract to P.pugio.

4.1.3.1. **Amino acids:** As has been stated above most of the workers have reported that substances of less than Ca.1000 molecular weight present in the tissues and their extracts are the principal stimulants eliciting feeding responses in the crustaceans. According to Carr and Gurin (1975) and Carr (1978) it is the amino acid fraction present in the human serum and extracts of blue crab, oyster, sea urchin and mullet elicit feeding response in the shrimp P.pugio. They reported that glycine, taurine, proline, glutamic acid and betaine present in the natural extracts and human serum function as response inducers. The attractiveness of ethanol extracts of the squid skin to the crab, Erimacrus isenbeckii was due to the presence of these amino acids only (Takei, 1977).

Amino acids and amines like L-glutamate, L-aspartate, L-arginine, glycine, taurine and L-alanine are present in the tissues and excretory products of many invertebrates upon which lobsters feed (Awapara, 1962; Kittredge et.al., 1962; Florkin and Schoffeneils, 1969; Takagi et.al., 1970; Konosu, 1971; DAniello, 1980, Suyama and Kobayashi, 1980) and the chemoreceptors are tuned to these amino acids that are most abundant in their prey (Derby and Atema, 1982c).

Several workers have reported that taurine is the most abundant Beta-amino acid in marine animals and is present at high concentration in the free amino acid pools of potential crustacean prey species (Wattanabe and Konosu, 1972; Fuzessery et.al., 1978a; Johnson and Ache, 1978; Carter and Steele,

1982; Carr and Derby, 1985; 1986a; Johnson and Atema, 1986; Smith et.al., 1987) and in zooplankton (Jeffries, 1969).

4.1.3.2. **Proteins and Peptides:** Peptides and proteins are also found to elicit behavioural responses in crustaceans (Carr and Gurin, 1975; Zimmer-Faust and Michel, 1980; Robertson et al., 1981). Heinen (1980) reported that some proteins when added to diets acted as incitant and ingestant. But he presumes that it is doubtful whether protein would function as a good feed attractant as they may not leach out rapidly enough to form a suitable odour trial.

4.1.3.3 **Nucleotides:** Several workers have reported that nucleotides function as feeding response inducers in different crustaceans (Carr et.al., 1984; Carr and Thompson, 1983; De Proenca, 1990). Structure activity relationships for nucleotides were studied by Carr and Thompson (1983). Their work and later by that of Carr et.al., (1984) showed that a denosine monophosphate (AMP) was a potent chemoattractant for Palaemonetes pugio. Best overall feed intake and assimulation were also reported obtained for the diet containing AMP proving it as a chemoattractant for M.rosenbergii also (Harpaz et.al., 1987b; De Proenca, 1990); but was a poor attractant for Pemaeus monodon (Hartati and Briggs, 1993). Many animo acids, trimethylamine and trimethylamine hydroxide (TMAH) stimulates feeding activity and increases feed intake in marine decapods (Laverack 1968) and also in M.rosenbergii (Costa-Pierce and Laws, 1985).

4.1.3.3. **Carbohydrates and Related Compounds:** Behavioural experiments have shown that carbohydrates, which are commonly found at high concentrations in algae and diatoms can be excitatory for certain crustaceans. Carbohydrate

would have a high signal to noise ratio for those animals which feed on foods containing high levels of carbohydrate (Trott and Robertson, 1984). These compounds have been found to stimulate feeding behaviours in crustaceans like; herbivores kelp crab, Pugettia producta (Zimmer et.al., 1979), filter feeder porcelain crab, Petrolisthes cinctipes (Hartman and Hartman, 1977), deposit feeder fiddler crab, Uca pugilator (Robertson, et.al., 1980; Robertson et.al., 1981), omnivore cray fish, Procambarus simulans (Ashby and Larimer, 1965), ghost crab (Trott and Robertson, 1984), krill (Hamner et.al., 1983) and kuruma shrimp, P.japonicus (Nakamura, 1987).

Among the saccharides, glucose is found to be stimulatory for crab U.pugilator (Robertson et.al., 1980) and among sugars cellobiose and galactose for P.japonicus (Nakamura, 1987). Heinen (1980), reported that mono sodium glutamate is a feeding attractant for crustaceans.

However marine crustaceans would find carbohydrates to be poor signals because they are generally present in high back ground concentrations in sea water (Burney and Seiburt, 1977). Shepheard (1974) also demonstrated a weak feeding response by crustaceans towards sugars when compared to amino acids.

4.1.3.4. **Aldehydes and Other Compounds:** Compounds like aldehydes, alcohols, amines and fatty acids were also found to function as chemostimulants in crustaceans (Shepheard, 1974; Takei and Ai 1971). Takei and Ai (1971) reported that P.japonicus preferred diets flavoured with materials like isobutyl aldehyde, hexyl aldehyde, ethyl butyrate and glutamic acid. However, Shepheard (1974) demonstrated that these compounds have only weak stimulatory property compared to amino acids.

4.2 SYNTHETIC CHEMICAL STIMULI

Behavioural and neurophysiological studies using a spectrum of stimulatory chemicals for crustaceans has been demonstrated by many authors Case, 1964; Ache et.al., 1976; Johnson and Ache, 1978; Heinen 1980). Low molecular weight nitrogenous substances like amino acids, nucleotides, amines and quarternary ammonium compounds were found to be most stimulatory for decapod crustaceans (Case and Gwilliam, 1961, Crisp, 1967; McLeese, 1970; Hindley, 1975; Allison and Dorsett, 1977; Carr, 1978; Ache, 1982; Bauer and Hatt, 1980; Carr and Derby, 1986a). While starch, sugars, alcohols, fatty acids and several other compounds have usually been given lesser responses or none at all.

However, studies in crustaceans have focussed mainly on the feeding stimulatory capacity of amino acids and like substances (Shelton and Mackie, 1971; Fuzessery and childress, 1975; Hindley, 1975; Derby and Atema, 1982a).

4.2.1. **Amino Acids:** The major chemical signal which is identified among aquatic animal are amino acids (Bardach, 1975 a and b), which are soluble and leach out rapidly to evoke feeding responses in crustaceans (Provasoli, 1976). Ache (1972) conducted a comprehensive investigation on the effects of amino acids and their various mixtures on the food searching activity of H.americanus. A wide variety of amino acids were found to elicit food seeking reaction at 10^{-6} to 10^{-5} M concentration. Amino acids were also reported to be an adequate stimuli for panalarid and homarid lobsters (Laverack, 1964; McLeese, 1970; Ache, 1972; Mackie, 1973; Shepheard, 1974).

4.2.1.1. **Taurine:** Several workers have stressed the importance of this amino acid as a feeding chemoreceptor stimulant in crustaceans (Case, 1964; Crisp,

1967; Ache, 1972; 1982; Shepheard, 1974; Carr and Gurin, 1975; Fuzessery and Childress, 1975; Allison and Dorsett, 1977; Bauer and Hatt, 1980; Heinen, 1980; Johnson and Atema, 1986). Taurine was effective both as a feeding attractant and as a mild feeding stimulant for P.monodon (Pascual, 1980; Hartati and Briggs, 1993) and P.japonicus (Deshimarus and Yone, 1978). This amino acid when incorporated in the diets of shrimps improved its palatability and acceptability.

4.2.1.2. **Glycine:** Deshimaru and Yone (1978) reported glycine as the most potent amino acid eliciting feeding response in P.japonicus. The acceptability of the diet and feeding rate increased after the addition of glycine in the diet. Heinen (1980) Bauer and Hatt (1980) and Bauer et.al., (1981) also reported glycine as a feeding stimulant for decapods as a whole.

4.2.1.3. **Glutamic Acid:** Glutamic acid ranks first after taurine among the most stimulatory amino acids for crustaceans (Heinen, 1980; Bauer and Hatt, 1980; Bauer et.al., 1981; Ache, 1982). Glutamic acid had been reported as a feeding stimulant for P.japonicus (Takei, 1969; Takei and Ai, 1971) and the most effective feeding response inducer in the lobster H.americanus (McLeese, 1973b).

4.2.2. **Other Chemical Stimuli:** Synthetic nitrogenous compounds like betaine, trimethyl amine, trimethylamine oxide and n-aminobutyric acid (Allison and Dorsett, 1977; Zimmer et.al., 1979; Allen et.al., 1975; Case, 1964; Laverack, 1963; Levandowsky and Hodgson, 1965; and Takei, 1977) and sugars like galactose and glucose are reported as adequate stimuli for crustacean chemorceptors (Archdale and Nakamura, 1992).

4.2.2.1. **Betaine:** The role of betaine as a feeding stimulant in decapod crustaceans were reviewed by Hashimoto (1967). Betaine is one of the major components of various tissue extracts (Carr, 1978) and plays a significant role in eliciting feeding response in crustaceans (Fuzessery and Childress, 1975; Carr, 1978; Bauer and Hatt, 1980; Bauer et.al., 1981; Carr and Derby, 1986; Harpaz et.al., 1987a; b; Harpaz and Steiner, 1990). Betaine produced feeding activity in P.pugio (Carr, 1978), cray fishes, crabs and lobsters (Hodgsons, 1958; Laverack, 1963).

4.2.3. **Species Specificity:** The specificity of amino acids and their effect on the food intake were studied in P.japonicus (Hashimoto, 1967; Takei, 1969; Takei and Ai, 1971; Deshimaru and Yone, 1978), P.merguiensis (Hindley, 1975), P.monodon (Murai et.al., 1981), Penaeus paulensis (Dos Santos Filho, 1983) Panulirus argus (Johnson and Ache, 1978) Palaemonetes pugio (Carr, 1978), M.rosenbergii (Farman-Farmaian et.al., 1979) Uca (Robertson, 1980) and Portunus pelagicus (Archdale and Nakamura, 1992). These studies indicated that species differed significantly in their specificity to different amino acids. Addition of glycine, to the diet significantly increased feed intake in P.japonicus, followed by an amino acid mixture, taurine, alanine and serine in the decreasing order, whereas aspartic acid, glutamic acid, proline and betaine hardly enhanced the palatability. In P.monodon glycine and betaine and in P.paulensis isoleucine improved the attractability and palatability of the diet. In the crab, P.pelagicus alanine, arginine, glycine, histidine, leucine, serine taurine and betaine and in Uca L-serine were identified to be stimulatory. The American lobster, Homarus americanus was responsive to amino acids like alanine, beta alanine, glutamic acid, proline, succinic and malic acid, arginine, glycine, taurine, aspartic acid and tyrosine (Mc Leese,

1970), but not to sugars, alcohols, nucleotides and nucleosides (Derby and Atema, 1982c).

L-glutamic acid was observed to be an extremely effective stimulant in Cancer antennarius and Cancer randall (Case, 1964) and L-glutamic acid, taurine and beta alanine in cray-fishes (Robins, 1959).

Most of the workers used L-isomers of amino acids, since they have generally been found to be a more effective stimulant in crustaceans than the corresponding D-isomers or a mixture of the two, in both neurophysiological (Case, 1964; Bauer et.al., 1981 Derby and Atema, 1982c) and behavioural studies (Mackie, 1973, Allison and Dorsett, 1977). But exceptions to the generalisation also exist (Case, 1964; Shepheard, 1974).

4.3. **TYPES OF FEEDING EFFECTORS:**

Based on the feeding response, the chemical feeding effectors have been classified by Lenhoff and Lindstedt (1974), as attractants, repellents, arrestants, incitants, suppressants, stimulants and deterrents. The same chemicals might sometimes perform all positive feeding functions in a crustacean but progressively higher concentration may be needed for action as attractants, arrestants, incitants and ingestants (Hindley, 1975; Heiner, 1980). They observed that some amino acids had threshold of less than 10^{-5} to 10^{-6}M as attractants but at higher concentration of 10^{-1} to 10^{-2} M act as incitant in penaeid shrimps.

4.3.1. **Feeding Inhibitors:** Some compounds inhibit filter feeding (Hartmann and Hartmann 1977) and reduces other feeding behaviours (McLeese, 1970; Allison and Dorsett, 1977; Zimmer-Faust et.al., 1984; Borroni et.al., 1985 when added into the medium). Proline had inhibitory effects on the feeding

behaviour of gulf weed shrimp, Leander tennuicornis (Johnson and Atema, 1986) and lobsters (Spalding and Atema, 1983; Johnson and Atema, 1985).

Leuandowsky and Hodgson (1965) found that some amino acids and amines which elicited feeding response in the spiny lobster P.argus at low concentrations, produced avoidance response at higher levels.

Crustaceans are sensitive to dopachrome, a component of octopus ink, which suppresses feeding behaviour (Kittredge et.al., 1974).

4.4. **POTENCY OF SYNTHETIC COMPOUNDS:**

Synthetic mixtures based on the composition of natural materials produced better response, but is always less than that of natural materials on which it is based (McLeese, 1970; Mackie, 1973; Ache et.al., 1978; Carr, 1978; Johnson and Ache, 1978). Hartati and Briggs (1993) measured the potency of some potential and commercially used feeding attractants for P.monodon. Taurine and an amino acid mixture designed to mimic a clam extract performed better in terms of feeding efficiency.

The amino acid component of shrimp extract accounted for about 60% of the total stimulatory capacity of the extract for antennular chemoreceptors in the lobster P.argus (Johnson and Ache, 1978). In another study of Mackie (1973) on the response of lobster H.gammarus to squid extract and synthetic mixtures, it was found that the mixtures of amino acids, betaine and TMAO were less than one half as effective as the whole extract. Synthetic mixtures of amino acids and betaine produced 60-100% of the activity of extracts of crab, oyster and sea urchin and only 30% of the activity of the mullet extract in P.pugio (Carr, 1978).

Carr and Gurin (1975) duplicated the feeding stimulation of human serum by a synthetic mixture containing serum proteins, amino acids, organic acids and other constituents in the shrimp P.pugio.

Comparing fractions and synthetic mixtures with their parent extract have demonstrated that less than 1000 molecular weight fractions were no better in their palatability than the whole extract from which they were derived, suggesting that other than amino acids there are palatability factors that adds to the total palatability of the whole extract (Carr, 1978; Carr et.al., 1984; Carr and Derby, 1986a).

4.4.1. **Effect of combinations on Feeding Activity:** Mixtures of substances can be more stimulatory than individual compounds, by additive or synergestic interactions and has been accounted in both behavioural and electrophysiological studies (McLeese, 1970; Shelton and Mackie, 1971; Mackie and Shelton, 1972; Mackie,1973; Beigler and Amen, 1976; Carr and Gurin, 1975; Fuzessery and Childress, 1975; Carr, 1978). Of particular interest is the potentiation in the attractiveness of amino acid mixtures when combined with betaine in P.pugio (Carr, 1978) and of tissue extracts and stimulant mixtures with cystine-HCl, lysine, glycine-HCI and methionine in H.americanus (McLeese, 1970).

According to Carr (1978), it may be worth while to add betaine to feeds, since it might be synergistic with dietary amino acids, or with one or more stimulatory amino acids.

Possible antagonism has also been reported, in which mixtures of very stimulatory substances become inferior to the individual components (McLeese, 1970; Johnson and Ache, 1978; Ache et.al., 1986). When used together proline,

alanine, arginine, and taurine acted antagonistically in lobster, H.americanus due to competitive interactions for receptor sites. Hartati and Briggs (1993) also reported that betaine alone is more attractive than glycine /betaine mixture for P.monodon at the same concentrations.

4.5. THRESHOLD LEVEL AND DOSE RESPONSE

Studies by Zimmer-Faust (1991) have shown some correlation between the ratio of chemical signals to the back ground "noise" and with the threshold level of detection in crustaceans. A "bell shaped" dose-response relationship in behavioural studies were obtained by synthetic attractants in the shore crab, Carcinus maenas (Shelton and Mackie, 1971) and towards increasing concentrations of betaine in P.pugio (Dahm, 1975; Carr, 1978). In the lobster H.americanus the response elicited by certain compounds showed no distinct correlation with the concentration (McLeese, 1970).

A wide range of amino acids elicits food seeking reaction at concentration as low as 10^{-5} to 10^{-6}M in H.americanus (McLeese, 1970) and $2x10^{-5}$ to 2×10^{-7}M in Portunus pelagicus (Archdale and Nakamura, 1992). Physiological studies in P.argus have show that taurine sensitive cells have response thresholds of about 10^{-10}M Fuzessery et.al., 1978; Ache et.al., 1988) with some cells being activated at concentrations as low as 10^{-13}M (Thompson and Ache, 1980). The actual dilution of taurine which elicit feeding response in M.rosenbergii is around 10^{-10}M (Derby and Harpaz, 1988) similar to that reported for P.argus. The response threshold of the glycine sensitive cells of P.argus is generally higher than those of the taurine sensitive cells and is approximately 10^{-6}M (Ache et.al., 1988) and the same for glutamic acid sensitive cells in cray fish is 2×10^{-5}M (Robins, 1959).

Data on optimum levels of some amino acids and cost consideration suggest that appropriate concentration of chemostimulants will be about 1% or lower (New, 1976). Dry diets might possibly require higher levels of chemostimulants than moist diets as drying apparently reduced the abilities of some foods to stimulate feeding behaviour in Crangon septemspinosa (Wilcoxon and Jeffries, 1974).

On combining the findings of several behavioural assays, it was found that the extracts of potential food organisms elicits arousal at less than picogram quantities of dry tissue per litre, walking and or searching at microgram quantities and food handling and or ingestion at milligram quantities (McLeese, 1973b; Pearson and Olla 1977; Mackie,1973).

4.5.1. **Factors Affecting Response Threshold:** Crustacean chemoreceptors are sensitive and can respond to a diverse array of chemical substances as well as to temperature and pH (Ache, 1982). It was found that the responsiveness of decapods to various chemical stimuli is affected by time of the year (Ennis, 1973), feeding state (Ache, 1982; Carr, et.al., 1984) and moulting state of the animal. (Harpaz et.al., 1987a). Threshold determination also varies with the way in which threshold is defined (eg. the minimum concentration to evoke 100% response versus the concentration for 50% response) and experimental design (eg. ascending versus descending concentration sequence).

The ecdysing chemoreceptor sensillae are least sensitive initially and gradually become sensitive with the progress of the moulting process (Guse, 1980). It was reported that apart from the brief duration of ecdysis (Stage E) the freshwater prawn M.rosenbergi remained responsive throughout its moult cycle (Harpaz et.al., 1987a). They stated that in response to very powerful

attractants M.rosenbergii skipped some of the intermediate feeding activity and reached the final stage much faster.

Several workers have studied the effect of feeding state on the responsiveness of P.pugio (Carr et.al., 1984), H.gammarus (Mackie and Shelton, 1972 and blue crab, Callinectus sapidus (Pearson and Olla, 1977). Apparent effectiveness of both synthetic and natural mixtures were found increasing with the degree of starvation. The detection and feeding thresholds of stimulants were also deceased several orders of magnitude after starvation Mackie and Shelton (1972) found that after 9 days starvation the feeding threshold of H.gammarus decreased from 10^{-4} to 10^{-6} g/litre of stimulant mixture.

5. CHEMORECEPTORS

5.1. DISTRIBUTION OF CHEMORECEPTORS

Interest in the chemical nature of the stimulants and the organization of chemoreceptor system is more recent. Several workers have investigated the distribution of chemosensory organs over the crustacean body surface (Laverack, 1968; 1975; Bardach 1975). Neurophysiological and behavioural studies have demonstrated that chemoreceptors concerned with feeding are located on many anterior appendages including antennules (Ache and Case, 1969; Ache, 1972; Shepheard, 1974); antennae (Tazaki and Shigenaga, 1974); mouth parts (Moller, 1978; Heinen, 1980; Ache, 1982) and legs (Case, 1964; Shelton and Laverack, 1968; 1970; Fuzessery and Childress, 1975; Hindley, 1975; Hatt and Bauer, 1980; Derby, 1982).

5.2. CHEMORECEPTOR TYPES

Chemoreceptors of aquatic crustaceans have been designated as low threshold or distance chemoreceptors and high threshold or contact ones, analogous to olfactory and gustatory receptors (Laverack, 1968; Shepheard, 1974). Dactyls that are lesser chemosensitive may function as contact chemoreceptors while the antennules with higher chemosensitivity may function as distance chemoreceptors (Fuzessery and Childress, 1975). But Derby and Atema (1982a) have shown that both leg and antennular receptors have lower thresholds.

The larvae of decapod crustaceans also possess well developed olfactory chemoreceptors capable of detecting amino acids and nitrogenous compounds of low molecular weight (Carr and Gurin, 1975; Heinen, 1980; Ache, 1982; Rebach, 1983).

5.3 CHEMORECEPTOR FUNCTIONS

Several workers have studied the function of chemoreceptor organs in feeding behaviour and spatial orientation of crustaceans (Hamner and Hamner, 1977; Derby and Atema, 1982b; Devine and Atema, 1982). The sergestid shrimp Acetes sibogue australis have receptors capable of precisely following prey odours (Hamner and Hamner, 1977). In the lobster H.americaus, the function of chemoreceptor organs in spatial orientation (Devine and Atema, 1982) and feeding behaviour (Derby and Atema 1982b) has been studied in detail. In decapods the antennular chemoreceptors are usually regarded as organs of smell (olfaction) and distinguished from dactyl and mouth-part chemoreceptors which are organs of taste (gustatory) (Atema, 1977; 1980; Heinen, 1980; Ache, 1982).

5.4 CHEMORECEPTOR SPECIFICITY

Crustaceans appear to have evolved specialised chemoreceptors to detect different chemical stimuli. (Carr, 1967; Derby and Atema, 1982b; Carr et.al., 1987; Ache and Carr, 1989). Carr et.al. (1987) provided an extensive description of crustacean chemosensory systems which documented specific chemoreceptors stimulated by purine and other nucleotides, taurine, betaine glutamic acid and glycine.

The chemical sensitivity of the crustacean receptors associated with the searching behaviour showed more selectivity to amino acids than to saccharides and other compounds (Case, 1964; Laverack, 1964; Nakamura, 1987). Chemoreceptor cells with selective sensitivity to specific amino acids are reported in H.americanus (Derby and Atema, 1982c, Johnson and Atema, 1983), crayfish (Derby and Harpaz, 1988) and crab, C.maenas (Schmidt and Gnatzy, 1989). The chemoreceptors are tuned to those amino acids that are most abundant in their prey than those that are nutritionally essential (Derby and Atema, 1982b). Specific uptake system for these amino acids are present in the olfactory sensilla of crustaceans (Gleeson et.al., 1987; Trapido-Rosenthal et.al., 1988).

Crustaceans appear to have evolved specialized chemoreceptors to detect taurine and electrophysiological studies have demonstrated receptors on the antennules and legs of lobsters extremely sensitive to taurine (Thompson and Ache, 1980) and have restricted response spectra (Fuzessery et.al., 1978a; b; Johnson and Atema, 1983; Johnson et.al., 1984). Beta alanine, structurally very similar to taurine is the second best stimulant for the taurine receptors of the spiny lobster, P.argus (Fuzessary et.al., 1978b).

Laverack (1964) and Laverack and Hodgson (1965) reported that antennular and dactyl receptors of P.argus are also sensitive to betaine, glycine and L-glutamic acid. Trapido-Rosenthal et.al., (1990) reported that the aesthetasc themselves contain large intracellular concentrations of taurine and glycine and these concentration being 10,000 fold greater than the response thresholds of chemosensory cells.

Crustaceans, molluscs and fishes also have receptors sensitive to betaine, a quarternary amine (Carr, 1967; Carr et.al., 1977). Dactyl chemoreceptors of the crab, C.maenas in sensitive to betaine at 0.01 to 0.001M concentration (Laverack, 1963) and also to L-glutamic acid at 10^{-6} M concentration (Case and Gwilliam, 1961).

5.5. CHEMORECEPTOR SITES

5.5.1. **Antennule:** The antennules have been implicated as important sites of chemosensory organs by several authors (Holmes and Homuth, 1910; Copeland, 1923; Laverack, 1964; Levandowsky and Hodgson, 1965; Hazlett, 1968; 1971; Ache, 1972; McLeese, 1972). Morphological, behavioural and electrophysiological studies have proved that the antennules are the main distance chemoreceptors of decapods used in locating food at a distance (Spiegel, 1927; Maynard and Dingle, 1963; Levandowsky and Hodgson, 1965; Ache and Case, 1969; Hazlett 1971a; 1971b; Ache, 1972, McLeese, 1973b; Shepheard 1974; Fuzessery and Childress, 1975).

In H.americanus, ablation of either lateral or medial filament of antennule impaired the chemosensory orientation, while ablation of both pairs greatly reduced orientation ability (McLeese, 1973b). Excitation of antennular chemoreceptors of P.argus by dilute chemical cues initiate search

activity and directs locomotion towards distant odour source (Maynard and Dingle 1963). In physiological studies, Ache (1973) and Shepheard (1974), activated the antennular receptors of lobster using low concentrations of organic compounds, which elicits feeding activity. Bell (1906) and Ameyaw - Akumfi (1977) have reported that there are evidences that freshwater cray fish do not rely greatly on antennules for distance chemoreception.

Although both the antennular filaments are chemosensory, behavioural evidence showed that only lateral filament is necessary for orientation to distant odour source (Schmitt and Ache 1979). Antennular chemosensitivity is ascribed to aesthetase sensilla borne on the lateral flagellum. However the medial filament which lacks the aesthetasc sensilla also apparently plays sensory role in crustacean chemoreception (Hodgson, 1958; McLeese, 1972; Hindley, 1975). The medial antennular filament of P.argus (Fuzesery, 1977), P.interruptus (Fuzessery and Childress, 1975) and Procambarus clarki (Hodgson, 1958) though lacking aesthetacs has also been found as chemosensory. Hodgson (1958) and Ameyaw-Akumfi and Hazlett (1975) stated that in freshwater cray fish the medial antennular flagellum alone is chemosensory, rather than the lateral one.

5.5.2. **Antennae:** Most of the workers stated that antennae have no feeding chemosensory role. But several workers like Holmes and Homuth (1910), Copeland (1923), Laverack (1964), Levandowsky and Hodgson (1965), Hazlett (1968; 1971) and Ache (1972) have implicated antennae as an important site of chemosensory organs.

5.5.3. **Walking Legs and Mouth Parts:** Behavioural and electrophysiological observations have indicated the presence of chemoreceptors particularly sensitive to amino acids on the dactylopodites (Case et.al., 1960; Case and

Gwilliam, 1961; Case, 1964; Shelton and Laverack, 1968; Hazlett, 1971b, Ai and Takei 1973b, Hindley, 1975; Lindsey, 1976; Derby and Atema, 1978; Hamilton, 1980), maxillipeds (Luther, 1930) and mandibles of crustaceans (Moller, 1978). Studies have shown that leg chemoreceptors have higher threshold than antennular receptors (Case and Gwilliam 1961; Ache, 1972; Shepheard 1974; Fuzzessery et.al., 1978a) and are thus considered as contact chemoreceptors (Fuzessery and Childress, 1975). Dactyl and mouth part chemoreceptors mediate high level stimulus detection close to or in contact with the stimulus source that elicits consummatory behaviour (Ache, 1982).

Luther (1930) described walking leg and maxilliped chemoreceptors, respectively as outer and inner contact chemoreceptors. On the walking legs the regions of greatest receptor density and specialization are the dactylus and propodus (Derby and Atema, 1982a). The chemoreceptors on the dactylopodities of the green crab, C.maenas. (Case et.al., 1960; Case and Gwilliam, 1961), C.antennarius and C.randall (Case, 1964) and crayfish Procambarus clarkii (Ameyaw-Akumfi, 1977) are highly sensitive to amino acids. According to Ache, 1982 dactyl chemosensitivity is usually ascribed to rows or tufts of setae that run lengthwise on each side of the dactyl.

5.6. **AESTHETASC SENSILLA**

Feeding response at the first process of feeding behaviour includes chemical recognition such as olfactory and taste reception (Scone, 1961) which is performed by the sensory setae present on the anterior appendages (Ache, 1982). The chemosensitivity of most of the chemoreceptor sites are usually ascribed to the presence of aesthetasc sensilla (Ache, 1982; Hodgson, 1958; Hindley, 1975). The distribution of chemosensory sensilla is best known from

the largest decapods (Hindley, 1975), where discrete clusters of chemoreceptors occur at multiple loci on the body and appendages such as antennules, pereopods dactyls and mouth parts, enabling descrimination between food particles and other materials. Fuzessery (1978) reported that aesthetasc chemoreceptors are fast adapting and suggested them to be the primary guidance component in chemosensory orientation. The non-aesthetasc chemoreceptors may serve to maintain search activity for a time in the absence of continued stimulation.

The aesthetasc hairs of the lateral flagellam of the antennules are implicated as being chemoreceptors by morphological (Laverack, 1964; Laverack and Adrill, 1965; Ghiradella et.al., 1968) electrophysiological (Ache, 1972; Shepheard, 1974) and behavioural studies (McLeese, 1970; 1973a; b; c; 1974; Snow, 1973a; Reeder and Ache, 1980). The thin walled aesthetasc hairs borne on the lateral antennular filament suggested its role in distance chemoreception by its morphological structure (Laverack, 1964; Laverack and Adrill, 1965; Ghiradella et al., 1968; Hazlett, 1971; Snow, 1973b; Shepheard, 1974).

Specific chemoreceptor sensilla on dactylus and propodus have been identified in crayfish (Hatt and Bauer, 1980) and in the lobsters, H.gammarus (Shelton and Laverack, 1968, 1970) and H.americanus (Derby, 1982). Structure of these setae suggested its role as contact chemoreceptors.

5.6.1. **Structure of the Sensilla:** Crustacean chemoreceptors are readily accessible for study because they occur in sensilla on the external appendages, primarily on the antennules and legs (Case, 1964; Laverack, 1964; Ache, 1982; Carr and Thompson, 1982). Carr et.al., (1987) studied the

structure and function of crustacean chemosensory system and provided an extensive description of crustacean chemoreceptors.

Dactyl (Lindsey, 1976) and antennal (Ball and Cowan, 1977; Dahl, 1973) setae that possess other morphological characteristic of chemosensors have a definite terminal pore when observed under Scanning Electron Microscope. Dactyl setae of various decapods take up dye at their distal tip, indicating they are permeable (Hamilton, 1980). While several workers propose the presence of pores in crustacean setae as associated with moulting or abrasion (Snow, 1974; Anderson, 1975; Guse, 1980; Hamilton, 1980). The external appearance of chemoreceptor sensilla may have been shaped by their microenvironments. For example, the antennular chemoreceptors remain in the water column, while the leg chemoreceptors are subjected to abrasion when the animal is walking or probing the substratum (Atema, 1980).

Materials and Methods

MATERIALS AND METHODS

Series of experiments were designed to evaluate the chemotactic activities of the shrimps, Penaeus indicus and Metapenaeus dobsoni and the chemotactic properties of various synthetic and natural compounds such as amino acids, sugars, nucleotides, tissue extracts and extract fractions.

The objectives were i) to evaluate the relative importance of feeding stimuli and ii) to investigate a) the nature of the stimulus which produce food seeking activity, b) the nature of stimulus which results in the mastication and ingestion of food and c) the mechanism by which the shrimps locate food following stimulation by food odour.

1. OUTLINE OF THE STUDY

1.1. STANDARD EXPERIMENTAL CONDITIONS

1. Shrimps were acclimated to the laboratory condition atleast for 5 days, before the trial.
2. Animals were deprived of food for 24 hrs prior to the trial.
3. Observation times were standardised occurring before 10.00 hrs and after 17.00 hrs.
4. Uniform water flow was maintained throughout the experimental tank.
5. Aeration was not provided during the trial.
6. Stimulus was assigned to the centre of the experimental chamber.
7. Rearing of shrimps and study was conducted at 20 ppt salinity.
8. Animals used for the study were healthy with complete appendages.
9. Animals were transferred to the acclimation chamber and conditioned for 12 hrs to the test condition when group response was studied and for 1 hour when studied individually.

10. Test was declared negative if no response occurred within 10 minutes.
11. Experimental tank was drained, and cleaned after each trial.
12. Animals once used for a test were discarded and for every new trial, fresh sets of animals were used.

1.2. BEHAVIOURAL DESCRIPTORS :

In order to document the response of the shrimps to test stimuli originating from the feed, a series of five critical behavioural response descriptors were developed, using feeding trials.

1. **Perception** : shaking of the exopodites of the 2nd and 3rd maxillipeds is evident along with extension of the pereopods. An intermittent flicking movement of the antenna occurs, pleopods are shaken strongly and the shrimps exhibit a searching activity. Perception or recognition of a chemical signal indicates stimulation of the chemosensory setae of antennules as well as those on the mouth parts and walking legs.

2. **Orientation** : characterised by increased searching activity with the pereopods accompanied by increased movement of pleopods in response to the detection of the chemical stimulus.

3. **Displacement** : Movement towards the stimulus source by walking or swimming.

4. **Arrival** : Arrival at the source of stimulus.

5. **Feeding activity** :
   a. Striking at the stimulus source with chelated pereopods.
   b. Ingestion

c. Rejection of the feed pellet or cessation of ingestion activity or leaving the stimulus source.

The first two behaviours are exhibited prior to searching, the last three are peculiar to feeding.

1.3. **RESPONSE DESCRIPTORS :**

Based on the property of the stimuli, to elicit the feeding response, they are classified as Lenhoff and Lindstedt (1974).

i) Attractant - a stimulus to which an animal responds by orienting toward the apparent source possibly over long distances.

ii) Arrestant - a stimulus that causes an animal to cease locomotion close to the apparent source.

iii) Repellent - a stimulus that causes an animal to orient away from the apparent source.

iv) Incitant - a stimulus that evokes initiation of feeding.

v) Suppressant - a stimulus that inhibits initiation of feeding.

vi) Stimulant - a stimulus that promotes ingestion and continuation of feeding.

vii) Deterrent - a stimulus that prevents continuation of feeding.

1.4. **EXPERIMENTAL ANIMALS**

Healthy post-larvae (15-20 mm), juveniles (30-50 mm) and sub adults (60-70 mm) of P.indicus and M.dobsoni were used for the study. P.indicus post-larvae were obtained from the hatchery and juveniles and sub-adults from the culture ponds. Post-larvae, juveniles and sub adults of M.dobsoni were collected from the backwaters. The animals were maintained in the laboratory with recirculatory system at 20±1 ppt salinity and pH between 8.0 to 8.4 on

standard feed. Animals were starved for 24 hrs. prior to the study to avoid the development of any behavioural preference for particular gustatory and olfactory stimulus.

1.5. METHOD OF EVALUATION

1.5.1. **Qualitative Evaluation** : Behavioural responses of shrimps to different stimuli were studied using the bioassay system described by Harada, et al (1982); after modification.

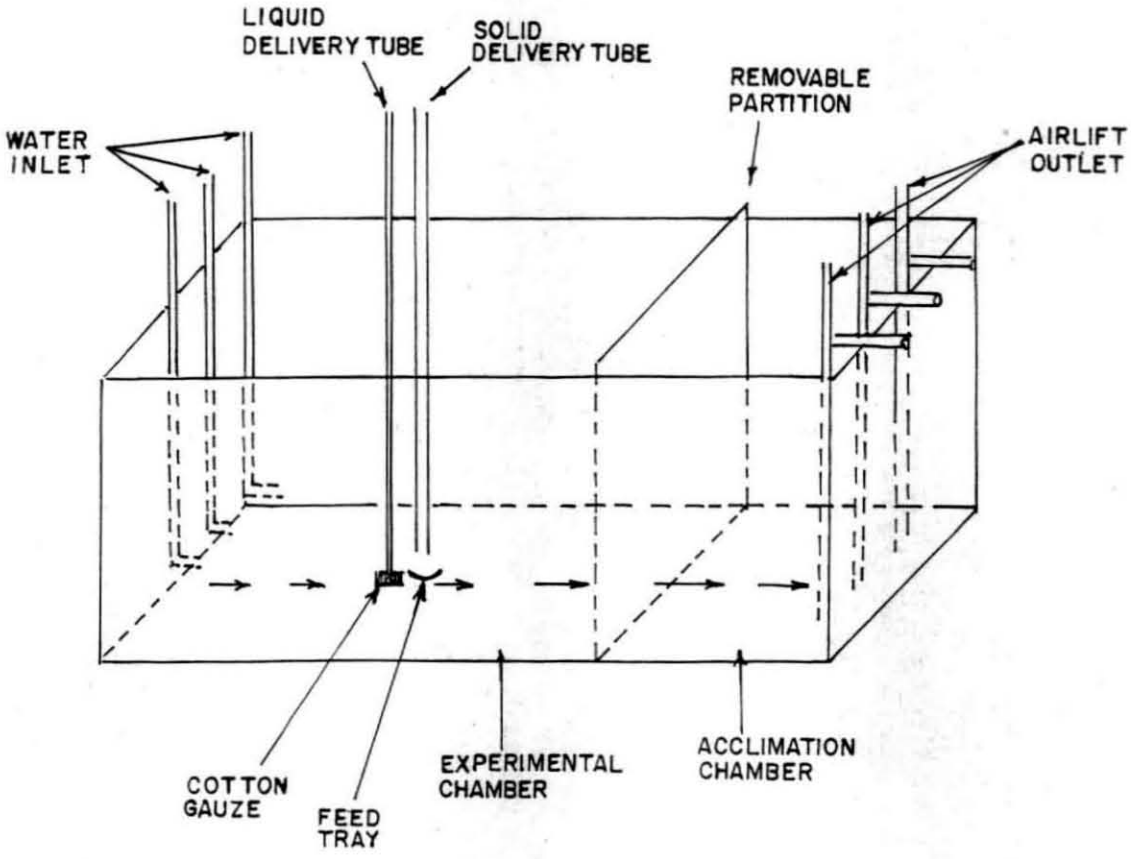
1.5.1.1. **Bioassay system** : The experimental system consists of a rectangular perspex tank (90 x 35 x 35 cm) divided into two unequal compartments of 60 and 30 cm long using a removable perforated shutter (Fig 1). The outer walls of the tank were covered black to minimise stress and disturbance to the animals during the trial. The larger compartment was used as the test chamber and the smaller one as the acclimation chamber to hold the experimental animal prior to the trial.

A gentle and continuous flow of aerated water was maintained by gravity flow through three inlet pipes placed equidistantly at the outer end of the test chamber during the course of acclimation and study. Three water lifts placed equidistantly in the acclimation chamber served as the outlet. The flow was maintained at a rate of 500 ml per minute.

A dye test was conducted to study the time required for the incoming water in the test chamber to reach the acclimation chamber and it was found to be around 30 sec.

Two removable delivery tubes, one for the introduction of liquid stimuli and the other for the solid stimuli, were fixed centrally in the first half of the experimental chamber. The former one consists of a graduated narrow

FIG 1 : Bioassay system used for the behavioural study.



glass tube with pointed delivery end penetrated into a cotton gauze of 5 x 5 x 2 cm size placed at the bottom of the tank. A 15 mm dia PVC tube with its delivery end open 5 cm above a watch glass at the bottom forms the the solid delivery system.

1.5.1.2. **Evaluation of Individual Response** : Behavioural responses of the shrimps to feeding stimulus were studied using subadult animals individually. Single animals were used in each trial to avoid possible group response when one shrimp first detected a feed stimulus.

The animals were introduced individually into the acclimation chamber and acclimated for an hour. Once the shrimp remained quiet the stimulus dose was introduced into the experimental chamber via the delivery tube. The feeding activities of the shrimp were recorded together with the time spent by the animals as they went through the various behavioural patterns. A responding shrimp will make an initial direction choice towards the stimulus source along the path of the central water current. The initial direction choice was recorded as either "+" or "_" with respect to the position of the stimulus source and the direction of displacement; or "no response" if searching activity was not elicited. The test was considered complete when the animal strikes the stimulus source or 10 minutes had elapsed since the introduction of the stimulus.

1.5.1.3. **Evaluation of Group Response :** The bioassay system described above was used for the study. Animals were transferred to the acclimation chamber and conditioned for 12 hrs. The test stimuli were injected into the experimental chamber and the partition was removed. Number of animals that exhibit positive feeding behaviour and strikes (locate) the stimulus source

with ingestive behaviour was noted at every 30 second interval for 10 minutes.

1.5.2. **Quantitative Evaluation**

1.5.2.1. **Bioassay System** : The fish activity recorder developed by Central Institute of Fisheries Technology (CIFT) was used to quantify the behavioural responses of shrimps. The instrument consists of a sensory unit and a recorder unit (Plates 1 and 2).

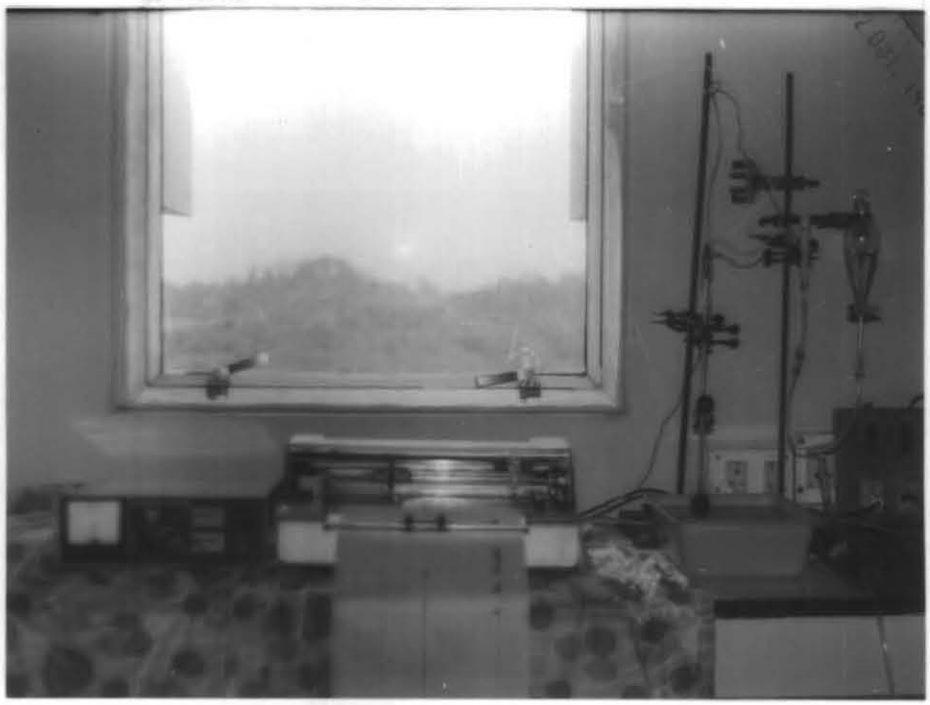
Sensory Unit : It consists of a two chambered perforated cage placed in a rectangular tray. The upper and lower chambers are separated by a perforated partition. The sensor was placed in the lower chamber, which senses the activities of the animals. The upper chamber was meant for holding the animals during the study, and was provided with a lid. The sensory unit was covered with a removable box.

Recorder Unit : Two types of recorders were used simultaneously ; (1) digital recorder (integrator) which displays the activity of animals as numerical units and (2) plotter, which recorded the activity of animals.

1.5.2.2. **Method of Study** : Sub adult shrimps were introduced individually into the upper chamber of the cage. A gentle and continuous flow of aerated water was maintained in the cage through an inlet tubing. The water in the tray was maintained at constant level with an outlet. Animals were acclimated to this condition for one hour and the recorders were pre-set to zero. Test stimuli was then injected into the cage through the inlet tubing without altering the flow and the behavioural responses of the animals were recorded.

PLATE I : The fish activity recorder developed by CIFT, Cochin used to measure the activity of shrimp.

PLATE II : Cage of the sensory unit with the shrimp in the upper chamber and sensor in the lower chamber of the Fish Activity Recorder.



1.5.3. **Evaluation of Ingestive Activity** : Feeding trials were conducted to quantify the ingestive activity of shrimps. Study was conducted in circular tubs of 40 litre capacity. Animals were fed ad libitum with the test diet. After 2 1/2 hrs the excess feed was collected and the quantity consumed was determined.

1.5.4. **Statistical analysis** : All experiments were designed statistically and the data were subjected to Friedman Rank Sum's Distribution Free Test (Hollander and Wolffe, 1973). Treatments were further compared using Distribution Free Multiple Comparison based on Friedman Rank Sums test.

2. PROCEDURE

2.1. RESPONSE OF SHRIMPS TO NATURAL STIMULI

2.1.1. **Preparation of the Test Sample** : The common protein ingredients used in shrimp feeds such as squid, shrimp, crab, black clam, green mussel, squilla, oyster and trash fish were used for the study. The test samples such as; whole tissue extract (TE), Lipid Free Extract (LFE) ; Protein Free Extract (PFE) and Free Amino Acid Fraction (FAA) were prepared as follows.

Whole Extract : The fresh tissue was weighed, ground well and extracted with cold distilled water by thorough stirring. The solution was filtered and centrifuged at low temperature to remove the suspended tissue particles.

2.1.2. **Evaluation of Chemotactic Behaviour of shrimps**

Protein Free Extract : The water soluble protein in the above stock solution was precipitated by adding methanol to a concentration of 75%. The precipitated proteins were removed by filtration through Whatman No.1 filter

paper. The filtrate was evaporated and then redissolved in distilled water to get test solutions of required concentration.

Lipid Free Extract : Chloroform was added to the stock solution and one volume of the aqueous solution was extracted twice with two volumes of chloroform.

Free Amino Acid Fraction : 80% ethanol was added to the tissue sample and homogenized thoroughly. The homogenate was placed in a water bath for 1 minute at 100°C to precipitate the protein; then centrifuged and the supernatant was collected. The residue was repeatedly extracted with 80% ethanol and thesupernatantswere combined and used. Free amino acids were determined according to the method described by A.O.A.C, 1990.

Free amino acid content of tissue extracts are given in Table 1.

2.1.2.1. **Test sample** : Squid tissue extract was used as attractant and squid ink extract as repellent sample. Tissue extract was prepared at 0, 0.5, 1.0, 1.5, 2.0 and 2.5 % (w/v) in filtered sea water. 1% tissue extract was used as a carrier medium for the repellent. The ink sac extract was prepared at 0, 0.5, 1.0, 1.5, 2.0 and 2.5 % in the carrier medium.

2.1.2.2. **Evaluation of Individual Response** : 10 ml of the test sample was injected into the gauze at a rate of 1 ml/minute, and the observations were made as described in section 1.5.1.2. Test samples were studied in the same animal from the lowest to the highest concentration. Each concentration was tested at 30 minute interval. Repellent and attractant samples were studied using new animals. Each treatment was repeated five times.

2.1.2.3. **Evaluation of Group Response** : This experiment was conducted using juveniles; as described earlier in section 1.5.1.3. 10 ml of the test sample

TABLE 1 : FREE AMINO ACID (FAA) CONTENT OF TISSUE EXTRACTS

TISSUE SOURCE	FAA LEVEL (ug/g tissue)
MUSSEL	497.1
SQUILLA	988.0
WHITE CLAM	554.75
BLACK CLAM	363.125
AMBASIS SP.	401.5
SHARK	609.15
EARTH WORM	917.5
CRAB	2320.0
OYSTER	532.5
SQUID	1480.0
CUTTLEFISH	350.0
SHRIMP	1320.0

was injected at a rate of 1 ml/minute. Each concentration was studied using the same set of animals starting from the lowest, at 30 minute interval. Each treatment was repeated five times using new animals.

The chemotactic index was estimated following linear regression procedures from the cumulative response. Regression coefficients were calculated for all treatment concentrations. The indices Db and Rb were calculated from the coefficients as follows.

$$Db = \text{Treatment coefficient} - \text{Control coefficient}$$

$$Rb = \frac{\text{Treatment coefficient}}{\text{Control coefficient}}$$

2.1.2.4. **Quantitative Evaluation** : Sub - adults of uniform size were used for the study. Test samples were injected into the cage one by one from the lowest concentration at 15 minutes interval. The same animal was used to test each concentration. Each trial was repeated five times using new animals, after flushing the system completely. For stimulant and repellent samples, seperate animals were used.

2.1.3. **Evaluation of Chemotactic Property of Tissue Components** :The property of major tissue components like lipids, water soluble proteins, and free amino acids were studied. Whole extract, Lipid Free Extract, Protein Free Extract, and Free Amino Acid Fraction form the test sample. The experimental set up and methods described in section 1.5. was followed here.

2.1.3.1. **Evaluation of Individual Response** : Experiments were conducted using subadults, where 1% (w/v) of extract types formed the test sample. Behavioural responses and the latency to elicit each was noted. Each treatment was repeated 5 times.

2.1.3.2. **Evaluation of Group Response** : Juvenile shrimps were used for the study. Test samples were prepared at 0, 0.5, 1.0, 1.5, 2.0 and 2.5 % (w/v) and the test was conducted as described earlier. Each concentration of the test sample was studied using the same set of animals. Coefficients, EC_{50} values (the lowest concentration required to elicit feeding response in 50% of the test animal in two minutes) and ET_{50} values (shortest time lag to elicit feeding response in 50% of animals at a given test sample concentration) were calculated following the linear regression procedure.

Potency and Percentage Activity of each fraction was calculated from the following equation,

$$\text{Potency} = \frac{EC_{50} \text{ of the test sample}}{EC_{50} \text{ of the whole Extract}}$$

$$\text{Percentage Activity} = \text{Potency} \times 100$$

2.1.3.3. **Behavioural Evaluation** : Behavioural intensity of shrimps were studied using 2.0% extract types as the test sample. This concentration was selected as it lies well above the range of EC_{50} values for all the test samples.

2.2. EVALUATION OF CHEMOTACTIC PROPERTY OF CHEMICAL STIMULI

2.2.1. Detection of Chemical Stimuli

2.2.1.1. **Test Stimuli** : Sugar and amino acid solutions of varying concentrations ranging from 10^{-9} M to 1M formed the test stimuli. These chemicals were dissolved in filtered seawater. The pH of the seawater was adjusted, if necessary, using dilute NaOH to dissolve the chemicals, higher

concentrations being omitted where the chemicals under test were not sufficiently soluble. Seawater was used since it was observed that the shrimp responded faster to seawater solutions than freshwater solutions.

2.2.1.2. **Method of Study** : The method followed was as described by Hindley, (1975). The animals (7-8 cm size) under test was secured dorsal side down in a groove in the wax block placed in a tray. The abdomen and pereopods were extended and immobilised. A capillary tube with a receiver fixed on a stand formed the delivery system. The position of the delivery tube was adjusted so as to deliver one or more drops of the test solution over the test appendage without disturbing the animal. Enough water was added to completely cover the animal. The animals were left undisturbed for at least 20 minutes before testing.

Each stimulus was tested by dropping three drops through the capillary tube over the mouthparts of the shrimp at 60 second intervals. After each drop, a positive or negative response was recorded. A positive response was one in which the shrimp made a vigorous movement of the mouthparts within 5 seconds of the introduction of the stimulus. Any other reaction was considered negative. If at least two drops out of three produced positive response it was considered that the shrimp was sensitive to the concentration under test. Since the lowest concentration used never produced a positive reaction, no separate control was used. Before each series of tests, and also after each chemical had been tested the shrimp were given one drop of standard squid extract 1% (w/v) over the mouthparts. If there was no positive reaction to this drop, the entire series of tests on that animal was disregarded. Seperate animals were used for each test chemical and then discarded. Each treatment was repeated 10 times.

The test solution was colored using a food dye to note the flow of stimulus over the shrimp and its subsequent dispersal. The drop contacted the mouthparts with little dilution by surrounding seawater. It was then rapidly dispersed by the respiratory current of the shrimp.

The lowest concentration of test stimuli which produced positive feeding response in 50% of the shrimp (EC_{50}) were obtained from the linear regression curves.

2.2.2. Behaviour of Shrimps to Chemical Stimuli

2.2.2.1. **Test Stimuli** : Test compounds of 10^{-1}M concentration were used individually and also in equimolar mixture. This concentration was selected as it lies well above the threshold concentration for both species.

2.2.2.2. **Method of Evaluation** : Behavioural responses of shrimps to chemical stimuli was evaluated using the bioassay systems and methods as described in section 1.5.

2.3. EFFECT OF WATER QUALITY PARAMETERS ON FEEDING RESPONSES

2.3.1. **Salinity** : The salinity levels studied were 5, 10, 15, 20, 25, 30 and 35 ppt. The animals were gradually acclimated to the test salinities and maintained at that level for a minimum period of 48 hrs. pH of the rearing and experimental water ranged between 8.0 and 8.4.

2.3.2. **pH** : The pH of the water was adjusted to the desired level by using either dilute Sulphuric Acid or Sodium bicarbonate and aerated well for 12 hours to remove excess CO_2. To avoid fluctuation in pH due to the activity of the animals a continuous flow of water with the required pH was maintained. The pH levels tested were 6.0, 7.0, 8.0, 9.0 and 10.0. Animals were gradually

acclimated to the test pH and maintained at that level for a minimum period of 48 hrs. pH below 6.0 and above 10.0 were not studied, since at pH 5.0 and 11.0 animals failed to feed and showed high mortality rates.

2.3.3. **Test Stimuli** : 10^{-1}M 1:1 Glycine : Betaine solution (AA), standard feed pellet (AI) and standard feed pellet coated with 1 M Glycine (AII) formed the test stimuli. The feed pellets were prepared following standard procedures. The percentage composition and proximate composition of the feed used are presented in Table 2 & Table 3. Crude protein was determined by kjeldahl analysis, crude lipid by soxhlet method, ash by combustion at 450°C for 12 hrs. and carbohydrate as the remainder of the dry weight. Moisture was determined by drying at 105°C for 24 hours, (A.O.A.C, 1990.)

2.3.4. **Method of Evaluation** : Behavioural responses and ingestive activity of the shrimps at the test conditions were evaluated qualitatively using the bioassay systems and methods described in section 1.5.

2.4. EFFECT OF STARVATION ON FEEDING BEHAVIOUR

The effect of varying degrees of starvation on the feeding responses of post-larvae, juveniles and sub-adults were studied as described in section 1.5. Animals starved for 0, 2, 4, 6, 8, 10 and 12 days formed the test group.

2.4.1. **Test Stimuli** : 1) 10^{-1}M (1:1 = Glycine : Betaine) solution and 2) Chemostimulant coated pellet.

2.5. LOCATION AND EVALUATION OF FEEDING CHEMORECEPTOR SITES

2.5.1. **Location of Chemoreceptor Sites:** Test animals were immobilized in the wax block and study was conducted as described earlier in the Section 2.III. In the case of appendages or body parts which occur close together, that part

TABLE 2 : PERCENTAGE COMPOSITION OF THE TEST DIET

INGREDIENTS	UNCOATED FEED	COATED FEED
FISH MEAL	15.0	15.0
SHRIMP MEAL	15.0	15.0
CLAM MEAL	15.0	15.0
GROUNDNUT OIL CAKE	20.0	20.0
RICE BRAN	20.0	18.5
TAPIOCA FLOUR	10.0	10.0
VITAMIN-MINERAL MIX	2.0	2.0
FISH OIL	3.0	3.0
ATTRACTANT (Glycine : Betaine=1:1)	-	1.5

TABLE 3 : PROXIMATE COMPOSITION OF THE TEST DIET

PROTEIN	-	40.53
CARBOHYDRATE	-	12.70
LIPID	-	8.26
CRUDE FIBRE	-	4.7
MOISTURE	-	6.7
ASH	-	10.91

or appendage other than the test organ, was ablated or coated with water proof enamel 24 hrs before the test, to avoid any erroneous response.

All tests were carried out with a 1% (w/v) squid extract as test stimuli. Three drops of the extract was introduced on to the part of the body being tested at two minute interval. After each drop a positive or negative response was recorded. A positive response was one in which the shrimp made a vigorous movement of the mouth parts within five seconds of the introduction of the test drop. Any other reaction was considered to be negative. In order to distinguish responses due to chemical stimulation from those due to mechanical stimulation by the drop or jet of tissue extract, the effect of drop or jet of seawater on the site concerned was also observed. If atleast two drops out of three produced positive response, it was considered that the site under test was having chemosensory role. After a test on one part of the body the animal was not tested for atleast 10 minutes. After a series of tests had been completed on an animal, a drop of standard squid extract was introduced over the mouth parts. If the animal did not respond with a vigorous movement of the mouth parts, all previous observations on that animal were ignored.

The test was repeated 10 times using new animals. Each site was tested randomly and no order of test was followed.

2.5.2. **Evaluation of chemoreceptor sites** : Healthy sub-adult shrimps with intact appendages were used for the study. The test appendage/body parts were prevented from exposure to the stimuli by coating 2-3 layers of a water proof enamel or ablated if needed, based on the treatment type. 24 hrs. was allowed following all treatments for recovery from the effects of coating and handling.

2.5.2.1. **Test Stimuli** : The stimuli used for this study were i) standard feed pellets ii) standard feed pellets coated with an amino acid mixture and iii) 10^{-1}M glycine solution for the behavioural evaluation using activity recorder.

2.5.2.2. **Treatment Groups** : Series 1 : Animals without 1) vision 2) antennae 3) antennule 4) pereopod, 5) pleopods 6) chelae of the first three pairs of pereopods and 7) mouth parts.

Series 2 : Animals with only ; 1) vision 2) antennae 3) antennule 4) pereopods 5) pleopods 6) chelae of pereopods 7) mouthparts 8) non - test appendages exposed.

2.5.2.3. **Method of Evaluation** : Behavioural response of the treatment groups were evaluated based on the feeding activities and the time lag to elicit each as described in section 1.5.

2.5.3. **Electron Microscopic Study** : To study the structural details of the appendages involved in chemoreception, Scanning Election Microscopy (SEM) was conducted. For SEM the mouthparts, antennule and pereopods were prepared by initial fixation in 2% gluteraldehyde in filtered seawater. Adhering debris and food material was removed by washing in filtered seawater, and again fixed in 2% osmium tetroxide for up to 12 hours. This ensured minimal shrinkage of the structures. The specimens were then dehydrated in graded acetone solutions and were then Critical Point Dried (CPD). After mounting they were coated with gold palladium vapour and examined in an Autoscan Electron Microscope.

2.5.4. **Statistical Analysis** : Wilcoxon's Paired Signed Rank Test was performed to compare each treatment group with the control. All treatment

groups together were subjected to Multiple Comparison based on Friedman Rank Sums Distribution Free Test.

2.6. PALATABILITY BIOASSAY :

Shrimps of 5.0 - 6.0 g, were used for the solid matrix ingestion bioassay. They were maintained and tested individually in glass aquaria of 10 l. capacity. To prevent any erroneous preconditioning to a particular flavour, the shrimp were fed in excess with diets containing the test materials used in the bioassay and the tanks were siphoned clean before the experiment.

The standard procedure of the bioassay was to present the test stimulus to the shrimp in an agar disc as described by Adams and Johnsen, (1986). The blank agar disc elicits little feeding activity by itself as demonstrated by very poor consumption in two-choice blank Vs. blank feeding tests. To reduce any possible effects of previous trials, tests were always conducted as two - choice experiments which allow direct comparison of consumption and preference between the two discs. To maximise the amounts ingested and for sufficient hardness so as to withstand the handling by shrimps 2.0% (w/v) agar was used for the block. The stimulus prepared was added to melted agar at 55-60^{0}C temperature and the agar master block cast into a petridish in which 3.5 cm. long plastic rods with a base were suspended. After the agar gets solidified they were cut using a disc cutter into 3 cm dia and 1.5 cm thick discs with the plastic rod. These rods were fixed to a mounting PVC pipe and this assembly lowered carefully into the experimental tank and fixed with a clamp and allowed to be fed for 4 hours. The agar discs were placed in a corner against the floor and wall of the aquaria so that the shrimp's rostrum prevented it from getting its mouthparts directly onto the discs shredding

them to pieces which would result in rendering the amounts actually consumed unmeasurable. In this case the shrimps used only the dactyls to pick off pieces from the agar disc and pass them into the mouth directly for ingestion, avoiding wastage. The positions of the disc were randomised for every test. The discs were weighed before and after the test period to determine the consumption and preference. A set of control discs were also placed in the water without the animal for four hours to determine the weight gain or loss due to hydration or dehydration of the disc. The blank agar disc prepared without stimulus served as the reference.

2.6.1. **Test Stimuli** : 1) Whole extracts (at 0.5, 1.0, 1.5, 2.0 and 2.5% levels of 15 tissue sources. 2) Whole extract, protein free extract, lipid free extract, free amino acid fraction, synthetic aminoacid and nucleotide mixture prepared at the same level as that of the free amino acid composition of the selected sources (Tables 4 and 5), 3) Amino Acids, 4) Nucleotides and 5) Sugars.

2.6.2. **Statistical Analysis:** The data were subjected to ANOVA and 't' test.

2.7. EFFECT OF ATTRACTANTS AND STIMULANTS ON FOOD INTAKE AND GROWTH

Feeding trials were carried out to study the effect of dietary supplementation of selected compounds on feed intake, assimilation, growth and survival of shrimps.

2.7.1. Laboratory Trials

2.7.1.1 **Study with semipurified diet** : Semipurified casein diets were used as the basal diet for testing the potential feeding attractants (Kanazawa et.al 1977). The percentage composition and proximate composition of the test diets are presented in Tables 6 and 7. The diets were supplemented with

TABLE 4 : PERCENTAGE COMPOSITION OF FREE AMINO ACIDS IN DIFFERENT TISSUE EXTRACTS.

AMINO ACIDS	CRAB	SHRIMP	SQUID	CLAM	FISH	OYSTER	MUSSEL
Alanine	7.304	11.250	7.589	8.525	8.870	13.300	8.380
Arginine	7.817	4.680	6.324	6.164	0.940	1.130	3.830
Asparagine	0.668	0.531	-	-	1.140	0.420	-
Aspartic acid	0.126	0.440	0.506	1.377	1.140	1.780	2.590
Cystine	0.411	-	-	0.328	-	0.240	0.410
Glutamic Acid	0.970	0.876	1.467	6.754	1.410	1.830	8.690
Glutamine	10.100	3.343	-	-	1.710	1.400	-
Glycine	38.804	49.721	24.790	21.574	10.240	8.700	6.620
Histidine	0.474	0.170	0.430	0.590	22.57	0.182	1.140
Isoleueine	0.365	0.562	0.784	0.721	0.470	0.073	0.830
Leucine	0.839	1.008	1.594	1.311	0.870	0.150	1.340
Lysine	0.776	0.260	0.810	1.639	3.960	0.600	5.170
Methionine	1.341	0.551	0.987	0.721	0.240	0.040	1.650
Phenylalanine	0.320	0.287	-	1.311	0.270	0.018	0.830
Proline	19.06	6.049	40.730	1.049	1.580	4.450	2.280
Serine	0.907	0.711	0.936	1.574	1.650	1.200	2.480
Taurine	6.448	17.510	9.36	43.54	38.280	60.130	47.160
Threonine	1.381	0.340	1.240	0.852	1.610	0.200	2.690
Tryptophan	0.451	-	-	-	-	-	-
Tryrosine	0.348	0.488	0.632	1.049	0.130	0.090	1.240
Valine	1.090	1.220	1.037	0.918	0.940	0.150	2.690
Ornithine	-	-	-	-	0.910	0.350	-
QUARTENARY AMMONIUM COMPOUND							
Betaine	48.995	45.280	42.540	67.900	51.240	91.070	97.090

TABLE 5 : PERCENTAGE COMPOSITION OF NUCLEOTIDES AND RELATED COMPOUNDS IN TISSUE EXTRACTS

NUCLEOTIDES	CLAM	SHRIMP	SQUID	CRAB	FISH	OYSTER	MUSSEL
Hypoxanthine	9.901	2.555	41.935	2.478	1.280	-	-
Adenosine	-	-	-	-	-	-	32.76
Inosine	28.713	0.666	22.581	5.081	18.320	7.780	46.55
Adenosine triphosphate	-	4.444	-	65.304	-	-	-
Adenosine diphosphate	5.941	15.888	-	13.507	0.180	5.550	-
Adenosine monophosphate	4.950	54.222	35.484	1.859	0.370	65.550	-
Inosine monophosphate	35.643	22.222	-	11.772	75.640	12.220	20.69
Uridine monophosphate	14.851	-	-	-	-	-	-
Guanidine monosphosphate	-	-	-	-	0.110	8.890	-
XMP	-	-	-	-	3.110	-	-

TABLE 6 : PERCENTAGE COMPOSITION OF THE SEMIPURIFIED DIET

	REFERENE DIET (REF)	TEST DIET
Casein	50.0	50.0
Sucrose	10.0	10.0
Starch	4.0	4.0
Glucosamine-HCL	0.8	0.8
Glucose	5.5	5.5
Na-citrate	0.3	0.3
Na-succinate	0.3	0.3
Fish oil	8.0	8.0
Cholesterol	0.5	0.5
Mineral mixture*	8.6	8.6
Vitamin mixture**	2.7	2.7
Alpha Cellulose	9.3	7.8
Attractant***	-	1.5

* Mineral mix (g/100 g of dry diet) K_2HPO_4 - 2.000, $Ca_3(PO_4)_2$ - 2.720, $MgSO_4$ $7H_2O$ 3.041, NaH_2PO_4 $2H_2O$ - 0.790.

** Vitamin mix (mg/100 g of dry diet) p amino benzoic acid - 10.00, biotin - 0.40 inositol - 400.00 nicotinic acid - 40.00, Ca-pantothenate - 60.00, Pyridoxine Hcl - 12.00, riboflavin - 8.00, thiamine - Hcl-4.00, menadione - 4.00, beta carotene - 9.60, - alpha tocopherol - 20.00, cyanocobalamine - 0.80, calcifirol - 1.20, Na - ascorbate - 2000.00, folic acid - 0.80, choline chloride - 120.00

***

Test diet		Attractant
(a) Based on the Free amino acid composition of tissue extract.		
SQA	-	Squid
SHA	-	Shrimp
CLA	-	Clam
CRA	-	Crab
(b) Selected L-amino acids/their mixtures.		
AA_1	-	L-glutamic acid, Glycine, Taurine, Betaine (1:1:1:1)
AA_2	-	L-serine, L-methionine, L-alanine, L-proline, Inosine (1:1:1:1:1)
AA_3	-	L-methionine, L-arginine, L-alanin, L-proline : Inosine (1:1:1:1:1)
AA_4	-	Taurine

TABLE 7 : PROXIMATE COMPOSITION OF THE SEMI-PURIFIED DIET

	%
Protein	42.8
Lipid	8.2
Carbohydrate	30.5
Ash	4.1
Fibre	6.2
Moisture	8.2

synthetic amino acids, nucleotides and their mixtures (@ 1.5%) based on the composition of selected tissue extracts.

Effect on feed intake : This study was conducted using juveniles of P.indicus and M.dobsoni. Ten shrimp were used as a treatment group with three replicates for each feed type. The shrimp were maintained in 30 l. tubs. The animals were fed ad libitum twice a day. The excess feed was collected 2 hrs after feeding and the faeces and exuviae were collected daily, washed with distilled water and dried at 60^{o} C. Aeration was provided throughout the trial and 50% water was changed daily. The feeding trial was conducted for a period of 12 days and the final weight of animals were determined. The attractiveness of each test diet was evaluated by the daily feed intake ratio.

Effect on growth : The effect of selected feed supplements on growth performance of P.indicus were conducted using juveniles. Each treatment group consisted of ten animals and the growth trial was conducted for 35 days. The feed trial was carried out as explained earlier. The ration was adjusted according to the feed consumption. The dead animals and exuviae were collected, weighed and recorded. The survival and gain in weight were recorded on every 7th day.

Study with compounded feeds : A series of growth trials were conducted in the laboratory using isonitrogenous feeds with fish meal, shrimp meal and clam meal as the protein source. The feeds were supplemented with 1.5% of the flavour mixture selected. The trial was conducted using P.indicus juveniles as explained earlier. The percentage composition and proximate composition of the test diets are presented in Tables 8 (a and b).

TABLE 8 b : PROXIMATE

Protein	
Lipid	
Crude fibre	
Ash	
Carbohydrate	
Moisture	

TABLE 8 a : PERCENTAGE COMPOSITION OF THE TEST DIETS USED IN THE GROWTH TRIALS UNDER LABORATORY CONDITIONS

Ingredient	FF_1	$FF_{2\&3}$	FS_1	$FS_{2\&3*}$	FC_1	$FC_{2\&3*}$	FD_1	$FD_{2\&3*}$
Fish meal	43.0	43.0	-	-	-	-	13.5	13.5
Shrimp meal	-	-	43.0	43.0	-	-	13.5	13.5
Clam meal	-	-	-	-	43.0	43.0	13.5	13.5
Groundnut oil cake	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Rice bran	20.0	20.0	20.0	20.0	20.0	20.0	22.5	22.5
Tapioca flour	1.0	9.5	11.0	9.5	11.0	9.5	11.5	10.0
Vitamin mineral mix	3.0	3.0	3.0	3.0	3.0	3.0	2.5	2.5
Cod liver oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Attractant/ Stimulant	-	1.5	-	1.5	-	1.5	-	1.5

* 2 - L-amino acid mixture based on the free amino acid composition of crab tissue extract.

3 - Mixture of L-arginine : L-alanine : L-proline : Inosine, L-serine : L-methionine (1:1:1:1:1:1:)

2.7.2. **Field Trial** : A multi-choice feeding trial was conducted in a 0.6 hectare grow-out pond to evaluate the effectiveness of dietary supplementation of selected attractants and stimulants on feed intake by P indicus under field conditions. Standard feed doughs supplemented with the selected compounds at 1.5% level (Table 9) were offered to the shrimps in feeding trays placed at the centre of the pond. Position of the dough was assigned randomly and allowed to feed for 30 minutes. The trial was conducted before 9.00 hours. Each treatments repeated three times, and the quantity of each dough consumed was determined.

Feed selectivity of shrimps were then evaluated using foraging ratio (FR) and index of selectivity (SI).

$$\text{Foraging Ratio} = \frac{S}{b} \quad \text{and}$$

$$\text{Index of selectivity} = \frac{S - b}{S + b}$$

where S = % of feed 'x' in the total feed consumed

b = % of feed 'x' in the total feed supplied

TABLE 9 : DOUGH TYPES WITH THE STIMULUS USED IN THE MULTI-CHOICE FEEDING TRIAL.

Dough Type	Test stimuli*	Dough Type	Test stimuli
F1	ASP	F17	Prol + Gly + Ala
F2	Ser	F18	Ala + Gly + Hist + Prol
F3	Tau	F19	Bet + Gly + Ala + Glut + Inos
F4	Arg	F20	Bet + Gly + Inos
F5	Meth	F21	Tyr + Phen + Lys
F6	Gly	F22	Tyr + Phen + Hist
F7	Bet	F23	Phen + Lys + Hist
F8	Prol	F24	Ala + Meth + Ser + Prol + Inos
F9	Ala	F25	Arg + Ala + Prol + Inos.
F10	Lys	F26	Glut + Asp + Gly
F11	Ala + Aspn + Glut	F27	Inos + Gly
F12	Ser + Ala + Aspn + Glut	F28	Prol + Ala + Leu
F13	Ser + Ala + Aspn + Glut + Bet	F29	Prol + Gly
F14	Arg + Phen + Isol + Leu	F30	Tau + Lys
F15	Gly + Arg + Ala + Prol	F31	Control
F16	Hist + Arg + Prol + Bet		

* Bet - Betaine, Gly - Glycine, Prol - Proline, Ala - Alanine, Arg - Arginine, Tau - Taurine, Glut - Glutamic acid, Ser - Serine, Aspn - Asparagine, Phen - Phenyl alanine, Isol - Isoleueine, Leu - Leucine, Hist - Histidine, Inos - Inosine, Lys - Lysine, Tyr - Tyrosine, Meth - Methionine, Asp - Aspartic acid.

RESULTS

1. RESPONSE OF SHRIMPS TO NATURAL STIMULI

1.1. CHEMOTACTIC BEHAVIOUR OF SHRIMPS

1.1.1. **Chemotactic Index:** The results of the study are summarised in tables 10 and 11 and Figs. 2a and b; which indicate the effect of varying concentrations of attractants and repellents on the chemotactic behaviour of shrimps. With the increase in concentration of attractant samples the percentage response and intensity of feeding behaviour increases, whereas in the case of repellent mixture, the same decreased sharply and differed significantly ($P<0.01$). The coefficients ('b' values) calculated from the percentage response increased with the concentration of the attractant (Fig. 3a) 3nd decreased with the repellent concentration (Fig. 3b). The coefficients for increased concentration was a positive value for the attractant and a negative value in the case of repellent.

All the Db values were above zero and increased with the attractant concentration; whereas in the case of repellents it was below zero and decreased with increase in concentration (Fig. 4).

The Rb values for the attractant were above 1 and increased with the increasing concentration (Fig. 5a). In the case of repellent the Rb values were below 1 and decreased with the increase in concentration (Fig. 5b).

From the above findings it is seen that an attractant sample will give a Db value always greater than zero and for the repellent it lies below zero. The zero Db value for a sample indicates neutral activity. An attractant sample will give an Rb value always greater than 1 and the repellent sample

TABLE 10 : PERCENTAGE RESPONSE* OF *PENAEUS INDICUS* AND *METAPENAEUS DOBSONI* TO ATTRACTANT CONCENTRATIONS.

	CONCENTRATION					
TIME	0.0	0.5	1.0	1.5	2.0	2.5
a. *P.indicus*						
30	2.56	19.68	31.23	41.15	57.54	68.21
60	2.61	27.21	56.27	91.13	98.72	112.54
90	3.05	39.93	79.31	121.95	151.03	187.03
120	3.15	50.13	99.67	141.23	193.65	251.27
150	3.17	80.16	121.07	179.13	216.21	291.54
180	3.17	121.06	159.63	191.20	231.57	318.07
b. *M.dobsoni*						
30	0.0	20.34	30.26	37.54	52.4	67.27
60	1.51	33.03	59.50	69.03	101.06	126.54
90	1.51	49.72	71.30	118.70	142.70	195.09
120	1.71	60.50	89.70	150.40	190.70	270.50
150	2.07	90.21	121.05	185.41	221.03	295.60
180	2.50	110.57	160.51	227.26	247.65	337.20

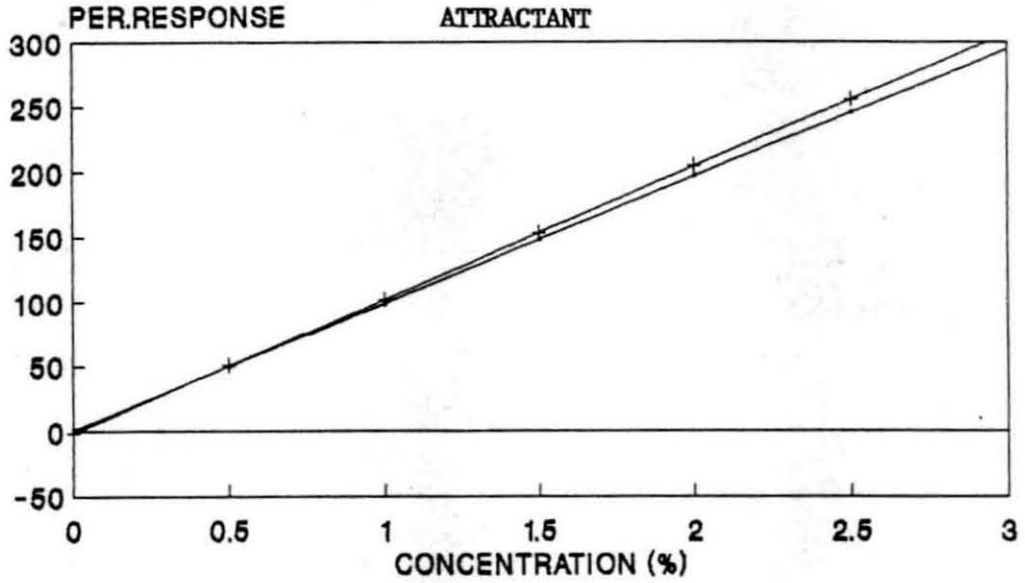
* Average cumulative values.

TABLE 11 : PERCENTAGE RESPONSE* OF PENAEUS INDICUS AND METAPENAEUS DOBSONI TO REPELLENT CONCENTRATIONS.

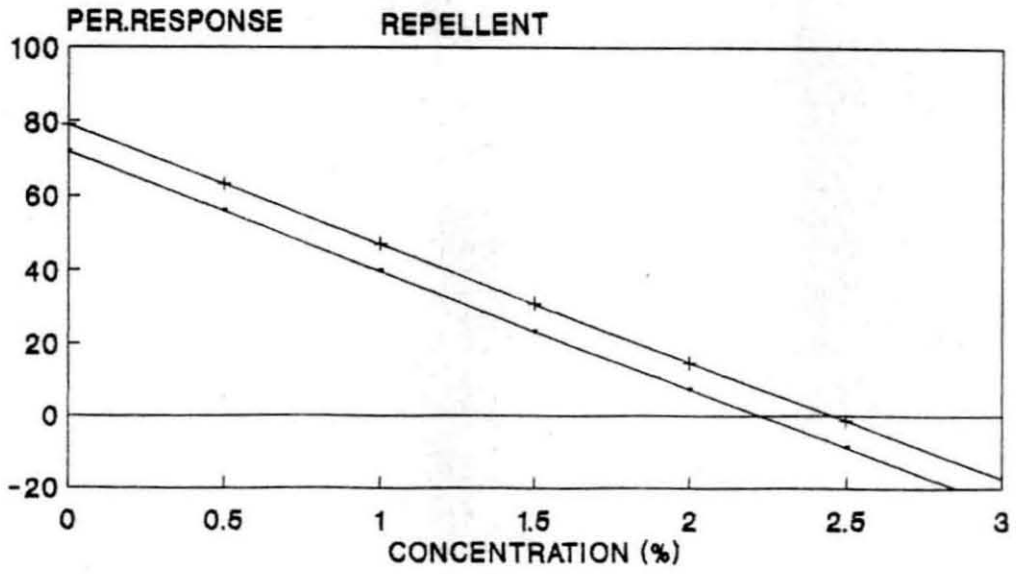
	CONCENTRATION					
TIME	1.0%	0.5	1.0	1.5	2.0	2.5
a. P.indicus						
30	31.23	20.13	5.26	5.51	7.04	2.96
60	56.27	23.33	15.21	10.21	8.54	4.94
90	63.31	23.11	15.21	10.21	8.96	4.94
120	99.67	39.73	19.17	16.14	8.96	5.87
150	121.07	42.73	23.05	20.07	10.75	5.87
180	159.63	49.57	33.99	20.07	12.96	7.63
b. M.dobsoni						
30	30.26	17.21	10.73	7.02	5.91	2.59
60	59.50	17.21	19.84	16.73	6.87	2.59
90	71.30	36.95	39.82	27.43	12.34	3.40
120	89.70	56.93	40.34	27.43	13.76	5.46
150	121.03	67.81	43.87	30.41	15.67	5.46
180	160.50	78.79	52.62	30.41	15.67	5.45

* Average cumulative values.

FIG 2 : The effect of varying concentrations of attractants and repellents on feeding responses in P.indicus and M.dobsoni.



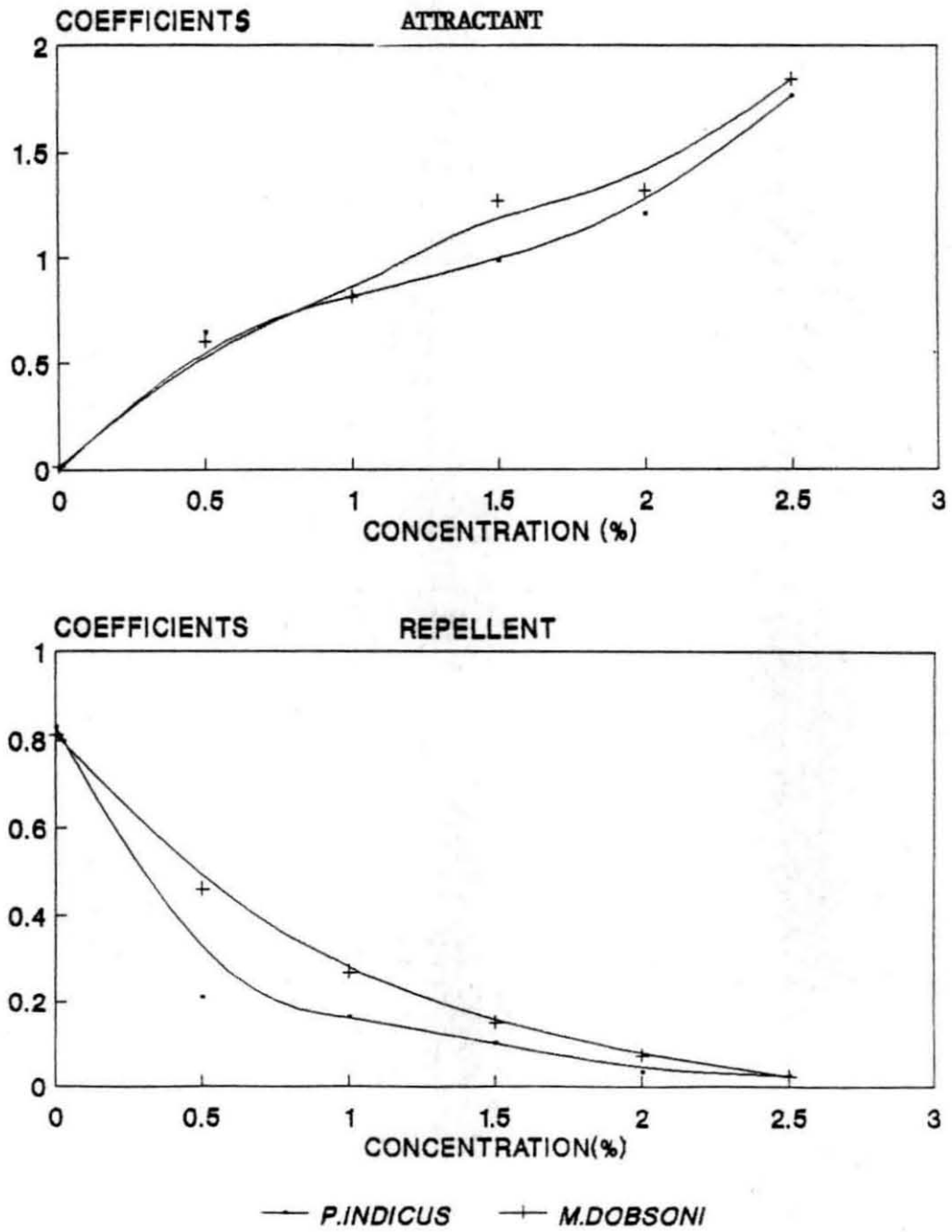
Y= 0.846+97.87 •X FOR *P.INDICUS* (r=.99)
Y=-0.98+102.6•X FOR *M.DOBSONI* (r=.993)



Y=71.9-32.25 •X FOR *P.INDICUS* (r=-0.852)
Y=79.24-32.18 •X FOR *M.DOBSONI*(r=-0.974)

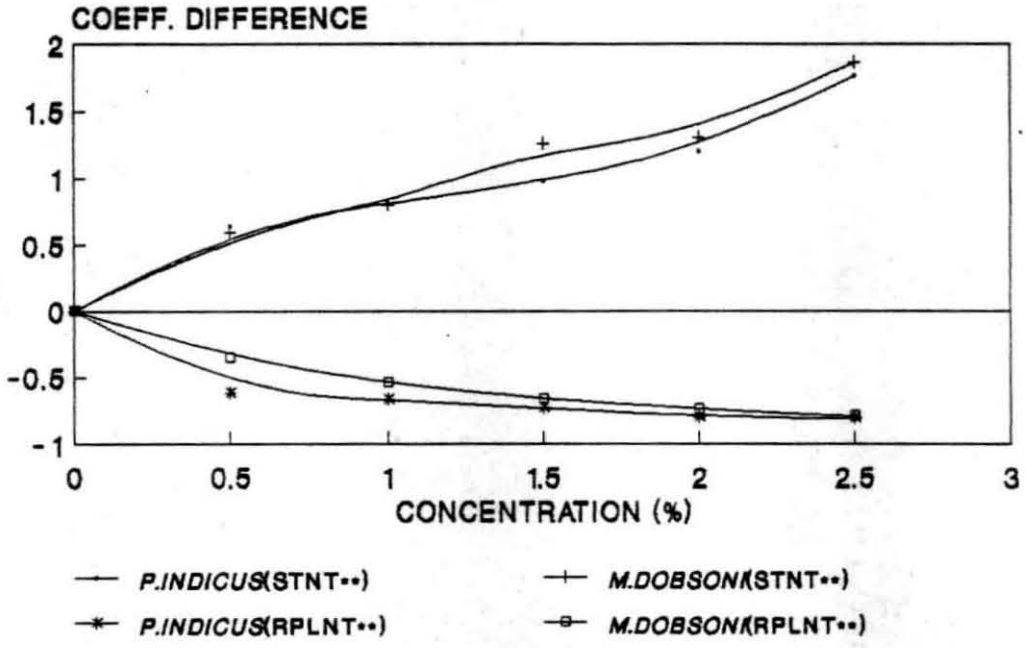
— *P.INDICUS* — *M.DOBSONI*

FIG 3 : Relationship between the stimulus concentration and the coefficients in P.indicus and M.dobsoni .



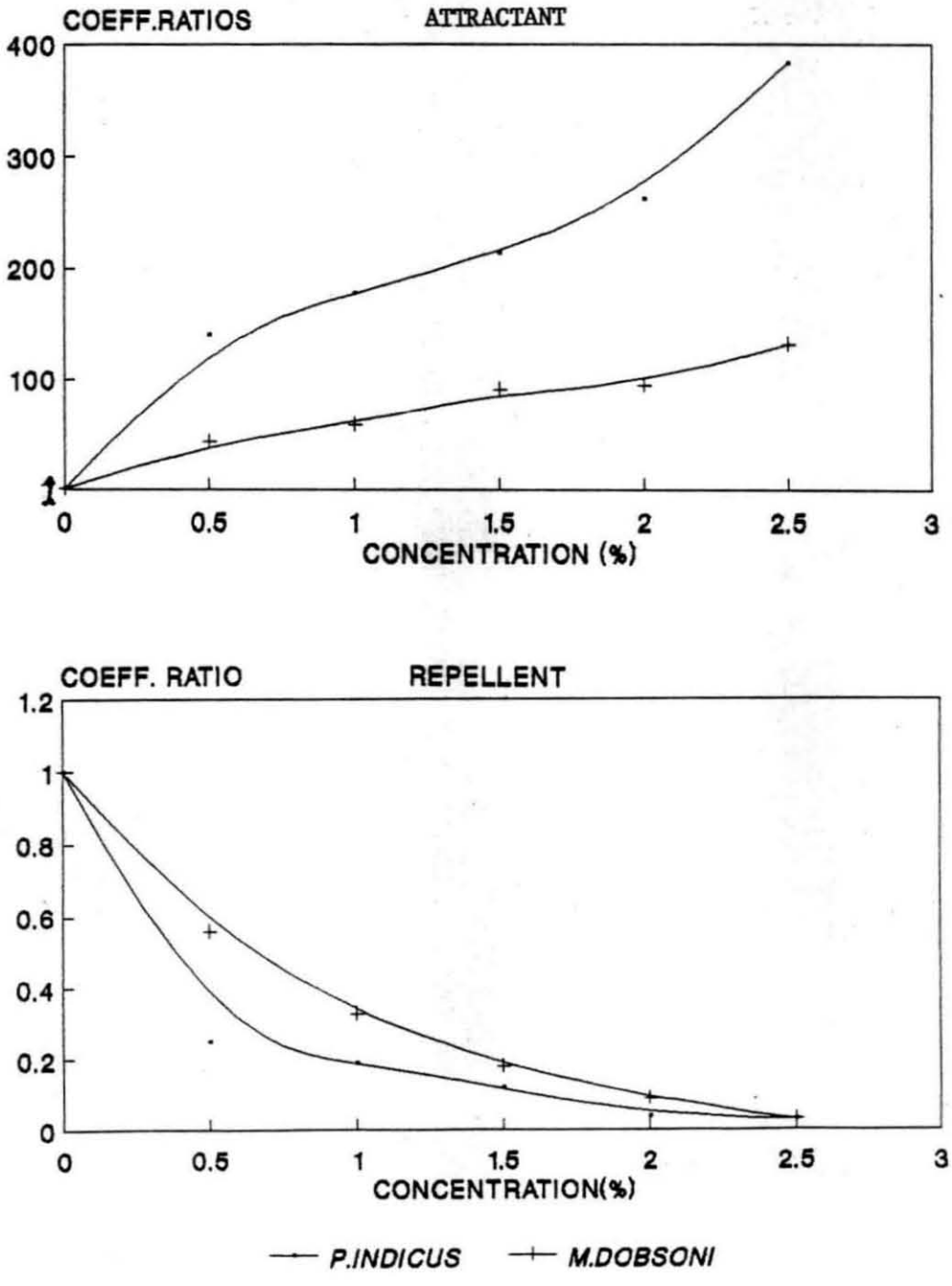
•,Coefficients were *calculated* using the cumulative percent*age response* values.

FIG 4 : Relationship between the stimulus concentration and the coefficient differences (Db) in P.indicus and M.dobsoni .



• Db = SAMPLE COEFF. - CONTROL COEFF.
•• STNT- ATTRACTANT RPLNT- REPELLENT

FIG 5 : Relationship between the stimulus concentration and coefficient ratios (Rb values) in P.indicus and M.dobsoni .



less than 1. Hence a positive Db value and an Rb value above 1 can be taken as the attraction index and a negative Db value and an Rb value below 1 can be taken as the repellent index to represent the chemotactic property of a sample, whereas the magnitude of the values can be used to assess the relative strength of the sample.

1.1.2 **Response of Shrimps to the Attractant:** The latency to elicit various feeding behaviours in shrimp, when attractant was introduced into the rearing tanks are given in Table 12. When sea-water was injected into the experimental tank no feeding behaviour was observed. But with the increase in the concentration of attractant from 0.5 to 2.5%, the time lag to detect or perceive the presence of a stimulus reduced from 80 to 26 second in P.indicus and from 90 to 30 seconds in M.dobsoni. The latency to locate or to arrive at the source of stimulus sharply decreased at higher concentrations in both the species. The latency to elicit the feeding behaviour decreased significantly with the attractant concentration ($P<0.01$).

Feeding behaviour was significantly more frequent in response to all concentrations of attractant sample than the control. Grooming increased significantly at the highest concentrations tested. Shrimps which entered the experimental chamber always followed the central current path while moving towards the gauze. The shrimp moved over the gauze and tried to pick it up using the chelate legs to be conveyed to the mouth and kept grooming over the gauze exhibiting typical ingestion behaviours. This behaviour continued for sometime, then they left the gauze and moved around the tank. But they returned to the gauze immediately and displayed feeding activities. In the case of control, no such feeding behaviour was observed. Occassionally only

TABLE 12 : TIME LAG IN SECONDS TO ELICIT FEEDING BEHAVIOUR IN PENAEUS INDICUS AND METAPENAEUS DOBSONI TOWARDS VARIOUS ATTRACTANT* CONCENTRATIONS.

BEHAVIOUR	CONTROL (0)	CONCENTRATION 0.5	1.0	1.5	2.0	2.5
a. P.indicus						
Perception	-	80	60	46	38	26
Orientation	-	95	70	53	45	30
Displacement	-	105	78	60	50	37
Arrival	-	140	108	85	70	55
Ingestion	-	145	110	90	75	60
b. M.dobsoni						
Perception	-	90	75	50	38	30
Orientation	-	100	80	55	45	35
Displacement	-	115	90	64	48	40
Arrival	-	145	110	85	68	58
Ingestion	-	150	115	90	75	53

* Squid extract

few animals moved towards the gauze, but they left the gauze immediately without any display of food seeking or feeding behaviour.

1.1.3. **Response of Shrimp to the Repellent:** The effect of repellent on time lag to elicit feeding behaviour is given in Table 13. When the repellent sample was mixed with the carrier medium the response time increased with the repellent concentration and at higher concentration both species showed no feeding behaviour, during the 10 minutes of observation.

In the case of repellent sample, the feeding behaviour decreased significantly with increase in repellent concentration and it was always lesser than that of control ($P<0.01$). Grooming declined and was zero at the highest concentrations for both species. In the case of control, the shrimps showed typical feeding behaviour as that of attractant sample. But on adding the repellent to the carrier medium, all the feeding activities declined initially and finally at higher concentration it reduced to zero. As the concentration of repellents increased, the animals avoided the central current path carrying the repellent and moved along the sides of the tank without exhibiting any feeding behaviour. As time increased all the animals left the acclimation chamber and the experimental chamber and congregated at the inlet position.

1.1.4. **Quantitative Response:** The results of the study are represented in Figs. 6 and 7 which indicates the intensity of shrimp behaviour when fed the attractant and repellent preparations of varying concentrations. The numerical measures of the behaviour are given in Table 14. This indicates that the feeding activity of shrimps depends on the intensity and nature of the stimulus. When the attractant mixture was given, a higher activity was recorded compared to the control. The activity recorded for the control was

TABLE 13 : TIME LAG IN SECONDS TO ELICIT FEEDING BEHAVIOUR IN *PENAEUS INDICUS* AND *METAPENAEUS DOBSONI* TOWARDS VARIOUS REPELLENT* CONCENTRATIONS.

BEHAVIOUR	CONCENTRATION					
	CONTROL (0)	0.5	1.0	1.5	2.0	2.5
a. *P.indicus*						
Perception	65	80	90	-	-	-
Orientation	70	95	135	-	-	-
Displacement	78	105	-	-	-	-
Arrival	100	135	-	-	-	-
Ingestion	105	-	-	-	-	-
b. *M.dobsoni*						
Perception	72	95	115	-	-	-
Orientation	80	105	125	-	-	-
Displacement	85	112	130	-	-	-
Arrival	115	145	-	-	-	-
Ingestion	120	-	-	-	-	-

Blank space indicates no feeding activity.

* Squid extract 1% + Squid ink

TABLE 14 : AVERAGE UNIT ACTIVITY RECORDED USING ACTIVITY RECORDER FOR VARYING CONCENTRATIONS OF ATTRACTANTS AND REPELLENTS IN P.INDICUS AND M.DOBSONI.

		STIMULUS CONCENTRATION					
		0	0.5	1.0	1.5	2.0	2.5
a.	Attractant						
	P.indicus	0	1.5	2.5	2.9	3.5	10.5
	M.dobsoni	0	1.0	2.0	2.7	2.9	9.7
b.	Repellent						
	P.indicus	2.3	1.5	0	0	0	0
	M.dobsoni	1.9	1.0	0	0	0	0

FIG 6 : Behavioural activity of P.indicus and M.dobsoni to varying concentrations of attractants recorded using Fish Activity Recorder.

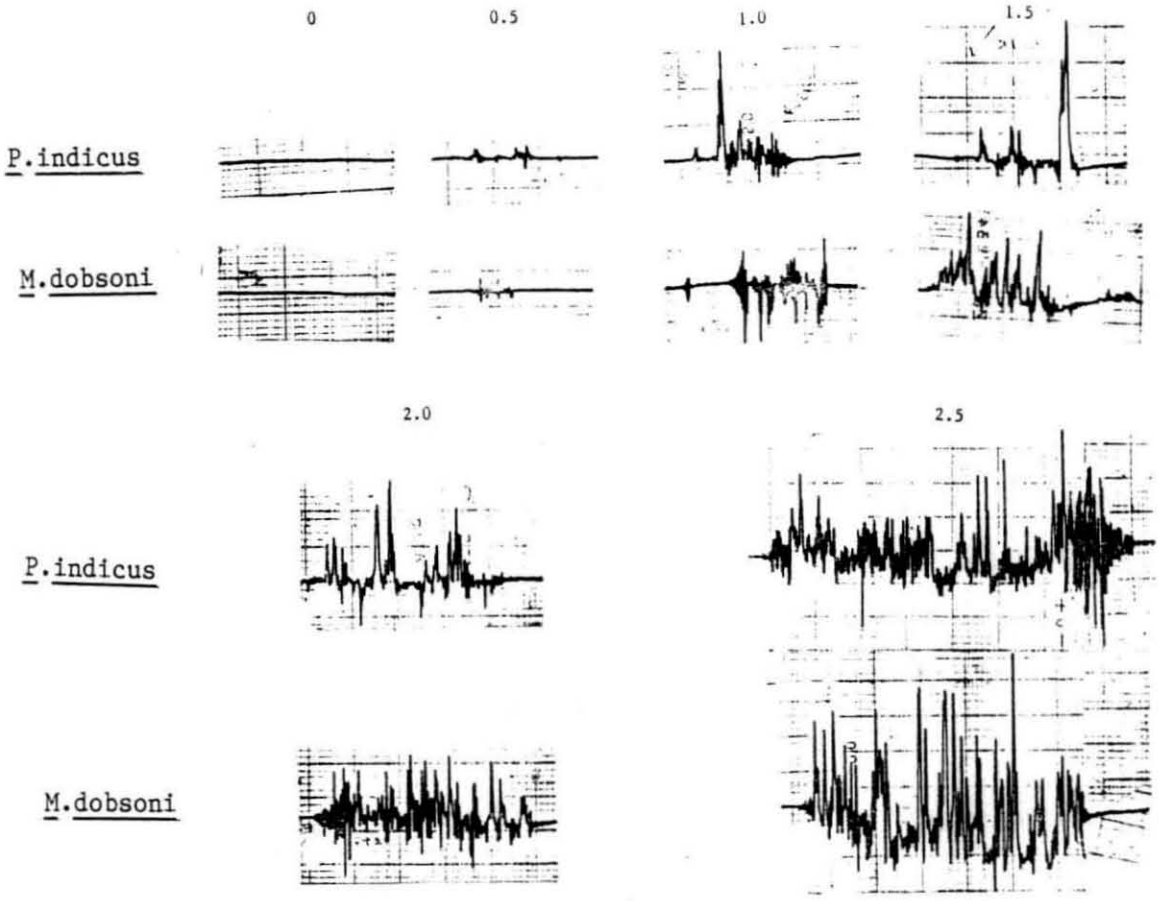


FIG 7 : Behavioural activity of P.indicus and M.dobsoni to varying concentrations of repellents, recorded using Fish Activity Recorder.

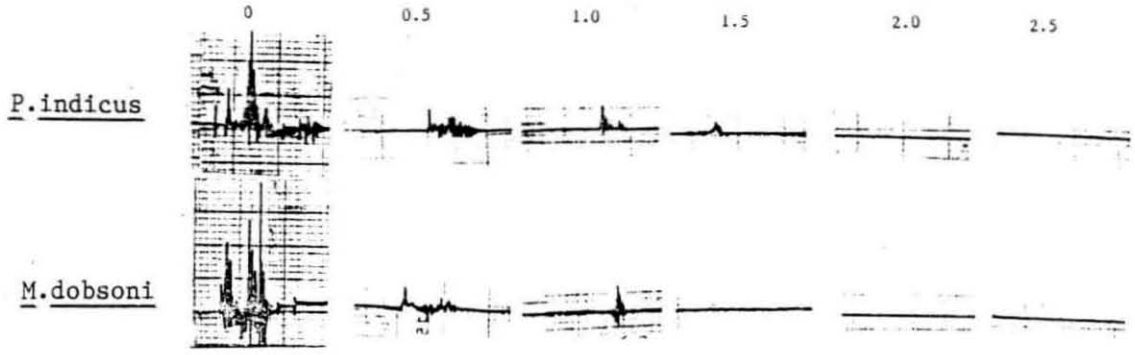


FIG 8 : Percentage response of P.indicus towards extract fractions of varying concentrations from different tissue sources.

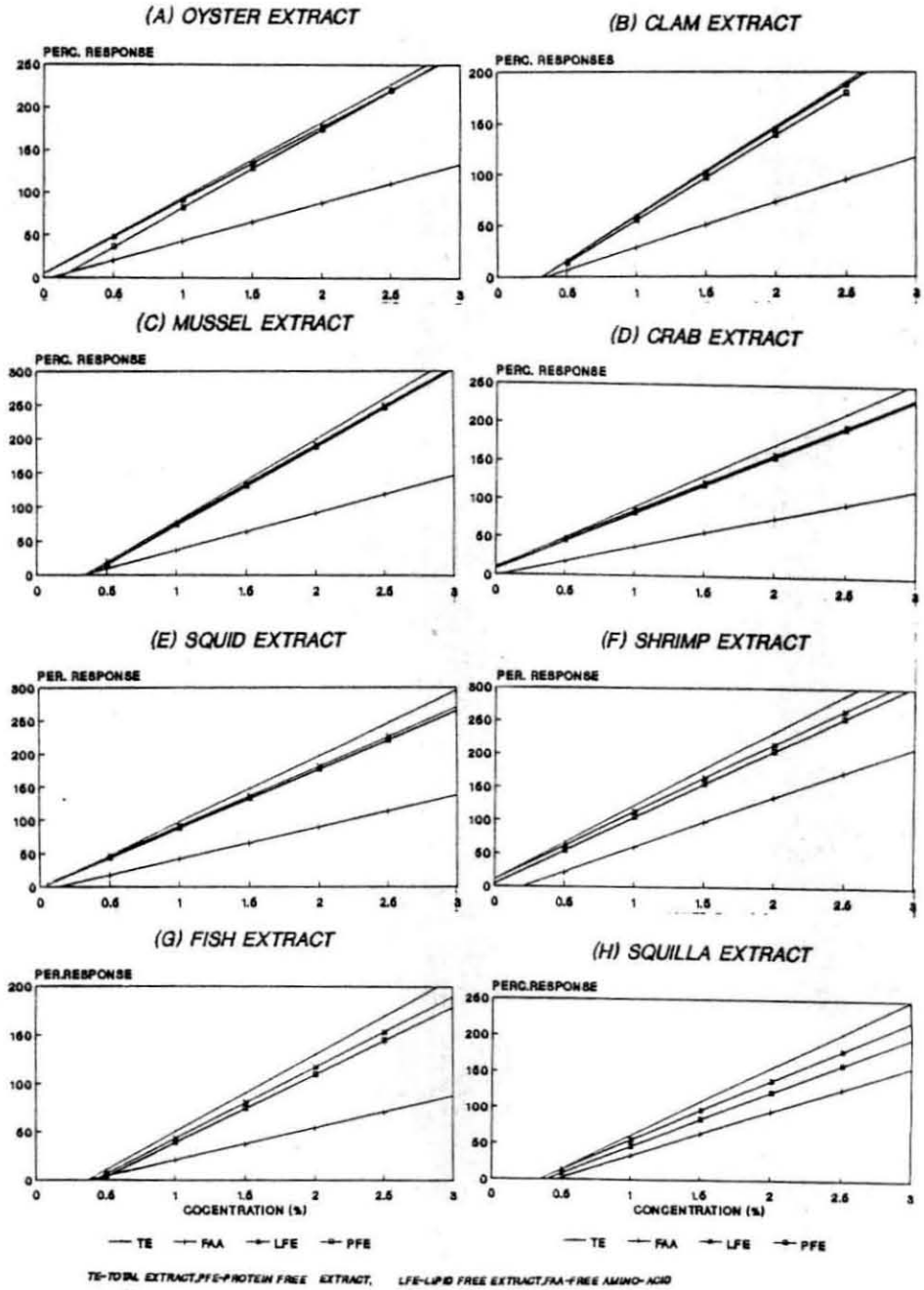
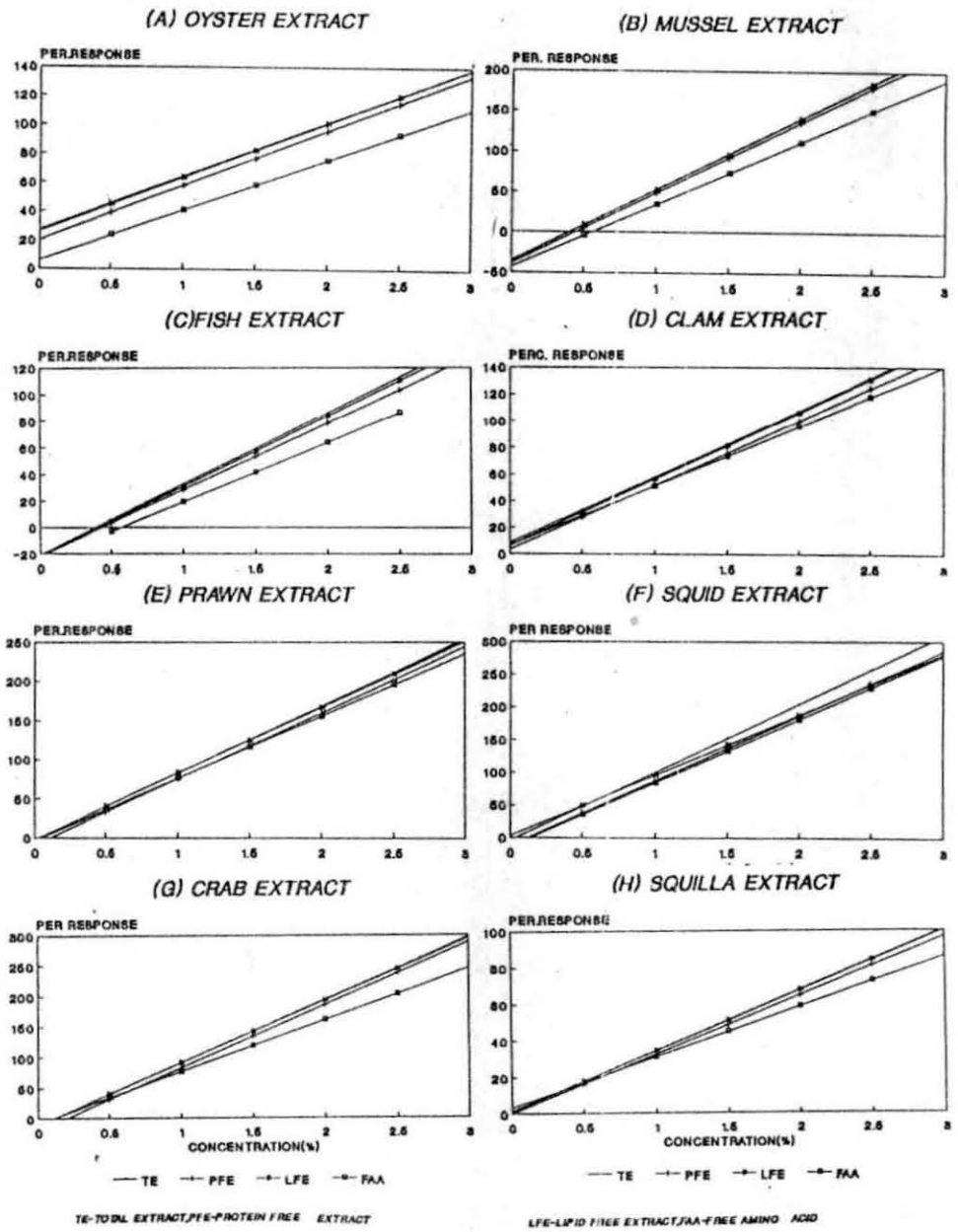


FIG 9 : Percentage response of M.dobsoni towards extract fractions of varying concentrations from different tissue sources.



differed significantly ($P<0.01$) in their ability to eliciting feeding response in shrimps. Both protein free extract and free amino acid fraction differed significantly from whole extract in their ability to elicit feeding response.

1.2.2. **Chemotactic Index:** The coefficients and the indices Db & Rb are given in Table 15 (a & b) for P.indicus and M.dobsoni respectively. It can be seen that all tissue samples and their extract types tested have an attractant property. The highest Db and Rb values were obtained for whole extract and the least for free amino acid fraction indicating that the whole extracts have the highest attractant property. The coefficients and index values obtained for different extract types differ significantly ($P<0.01$) indicating the role of different tissue components in eliciting feeding responses.

1.2.3. **Effective Concentrations (EC_{50}):** The EC_{50} values of different tissue extracts and extract types for P.indicus and M.dobsoni are given in tables 16 (a & b) respectively. The smallest EC_{50} values were obtained for whole extracts among the extract types tested. On removal of the protein fraction alone and both the lipid and protein fraction together led to a significant increase ($P<0.01$) in the EC_{50} values indicating a reduction in its chemotactic property. Removal of the lipid fraction alone resulted only in a nominal increase in EC_{50} values. The EC_{50} value of whole extract ranged between 0.33 and 0.99 for M.dobsoni and 0.52 and 1.48 for P.indicus. EC_{50} values of PFE range between 0.462 and 1.170 for M.dobsoni and 0.635 & 1.58 for P.indicus and of FAA; between 0.833 & 1.875; and between 0.673 and 1.69 for M.dobsoni and P.indicus respectively. Among the tissue types tested the lowest EC_{50} value was obtained for squid extract followed by crab and oyster extract and the highest value for squilla extract in P.indicus. But in M.dobsoni the lowest EC_{50} values were in prawn, squid and crab in order and highest for

TABLE 15 a : CHEMOTACTIC INDICES OF VARIOUS TEST SAMPLES FOR PENAEUS INDICUS AND METAPENAEUS DOBSONI

		OYSTER	MUSSEL	FISH	CLAM	PRAWN	SQUID	CRAB	SQUILLA
a. P.indicus									
TOTAL	b	88.68	120.0	83.32	86.70	110.80	99.25	81.96	93.74
EXTRACT	Db	88.675	119.995	83.315	86.695	110.795	99.245	81.96	93.735
	Rb	19278.26	26086.96	18113.04	18846.80	24085.96	211575.1	17876.39	20377.26
PROTEIN	b	85.99	114.01	72.34	83.26	109.23	98.05	77.79	73.46
FREE	Db	85.985	114.005	72.335	83.255	109.225	98.015	77.75	73.455
EXTRACT	Rb	18692.50	24785.96	15725.08	18099.00	23744.65	21307.7	16909.87	15968.50
LIPID FREE	b	87.245	114.54	78.24	85.92	110.70	98.65	81.82	82.71
EXTRACT	Db	87.235	114.535	78.235	85.915	110.695	110.695	81.8154	82.705
	Rb	18969.22	24899.0	17007.7	18677.26	24065.2	21442.48	17789.96	17979.93
FREE AMINO	b	45.26	55.14	36.26	43.99	74.89	31.02	38.61	61.92
ACID FRACTION	Db	45.255	55.135	36.255	43.985	74.885	31.015	38.605	61.915
	Rb	9838.13	11985.96	7881.61	9563.0	16279.43	6742.48	8392.48	13459.80

* Control b = 0.004.

TABLE 15 b : M.dobsoni

EXTRACT TYPE	INDEX	OYSTER	MUSSEL	FISH	BLACK CLAM	PRAWN	SQUID	CRAB	SQUILLA
TOTAL	b	37.02	88.0	53.32	47.84	85.340	104.24	102.24	33.11
EXTRACT	Db	37.006	87.986	53.306	47.826	85.326	104.226	102.476	33.096
	Rb	2644.286	6285.714	3808.571	3417.142	6095.714	7445.714	7320.714	2365.0
PROTEIN	b	37.68	87.12	49.94	46.54	83.98	99.12	102.0	32.28
FREE EXTRACT	Db	37.666	87.106	49.926	46.526	83.966	99.106	101.986	32.266
	Rb	2691.429	622.857	3567.143	3324.286	5998.57	7080.0	7285.711	2305.71
LIPID FREE	b	37.29	87.85	52.80	47.50	84.15	103.88	102.18	33.07
EXTRACT	Db	37.276	87.836	52.786	47.486	84.136	103.866	102.166	33.056
	Rb	2663.57	6275.0	3771.43	3392.86	6010.71	7420.0	7298.57	2362.14
FREE	b	34.67	77.28	44.72	42.10	79.46	98.99	84.71	27.63
AMINO ACID	Db	36.656	77.266	44.706	42.086	79.446	98.976	84.696	27.616
	Rb	2476.43	5520.0	3194.286	3007.143	5675.714	7070.714	6050.714	1973.71

* Control b - .016

TABLE 16 : EFFECTIVE CONCENTRATION (EC_{50}) OF DIFFERENT TEST STIMULI REQUIRED TO ELICIT FEEDING BEHAVIOUR IN PENAEUS INDICUS AND METAPENAEUS DOBSONI.

TISSUE TYPE	TOTAL EXTRACT (TE)	PROTEIN FREE EXTRACT (PFE)	LIPID FREE EXTRACT (LFE)	FREE AMINO ACID FRACTION (FAA)
a. P.indicus				
Oyster	0.610	0.790	0.650	1.230
Mussel	0.950	1.030	0.970	1.220
Fish	1.310	1.430	1.370	1.690
Clam	0.830	0.980	0.870	1.010
Prawn	0.633	0.700	0.637	0.713
Squid	0.520	0.635	0.529	0.673
Crab	0.610	0.710	0.623	0.720
Squilla	1.480	1.580	1.510	1.690
b. M.dobsoni				
Oyster	0.510	0.670	0.530	1.170
Mussel	0.750	0.875	0.789	1.275
Fish	0.990	1.170	1.130	1.875
Clam	0.890	0.965	0.920	1.490
Prawn	0.330	0.467	0.367	0.833
Squid	0.503	0.570	0.535	1.133
Crab	0.533	0.620	0.545	1.370
Squilla	0.843	1.067	0.950	1.310

fish extract. The EC_{50} values of tissue samples and extract types differed significantly in both species ($P<0.01$).

1.2.4. **Latency to Respond:** The time lag between the stimulus introduction and the display of feeding behaviour are given in Tables 17 and 18. Among the various test stimuli, squid extract elicited the positive feeding behaviour at the shortest time (30 secs) in P.indicus (Fig. 10 and 11). Where as M.dobsoni responded more quickly within 25 seconds to shrimp extract . The removal of the different components from the extract increased the time required to elicit feeding behaviour.

The time required to locate the source of stimulus by 50% of the test animals (Et_{50}) when different test stimuli were introduced are given in Table 19. Among the various test samples, P.indicus elicited immediate response and located the stimulus source within 55.69 seconds, when whole squid extract was provided, whereas M.dobsoni responded immediately and located the source in 42.09 seconds towards the shrimp extract. Among the test stimuli, whole extracts elicited immediate feeding response followed by lipid free extract protein free extract, and free amino acid fraction. All test stimuli studied differed significantly in their Et_{50} values in both species ($P<0.05$). Removal of various components from the tissue extracts, significantly reduced its effectiveness, as evident from the increased time lag for eliciting the feeding response and large Et_{50} values.

1.2.5. **Potency of Test Samples:** The potency of different tissue types and their extracts are presented in Table 20 for both the species. The removal of different components like proteins and lipids from the tissue extract led to a significant reduction ($P<0.01$) in their potency. The potency of Protein Free Extract ranged between 0.772 and 0.937 in P.indicus and between 0.761 and

TABLE 17 : TIME TAKEN BY THE SHRIMPS TO DETECT THE STIMULUS SOURCE.

PERCEPTION

TISSUE SOURCE	TE	LFE	PFE	FAA
a. P.indicus				
Oyster	45	50	65	85
Clam	55	55	70	95
Mussel	50	55	60	85
Fish	80	95	115	135
Prawn	40	45	60	75
Squid	30	40	55	60
Crab	43	45	65	80
Squilla	80	90	105	125
b. M.dobsoni				
Oyster	55	85	55	115
Clam	70	120	95	135
Mussel	65	95	80	120
Fish	85	120	100	150
Prawn	25	40	30	55
Squid	30	50	30	70
Crab	45	55	50	70
Squilla	85	120	105	165

TABLE 18 : TIME TAKEN BY THE SHRIMPS TO LOCATE THE STIMULUS SOURCE.

INGESTION

TISSUE SOURCE	TE	LFE	PFE	FAA
a. *P.indicus*				
Oyster	80	95	110	135
Clam	85	105	98	135
Mussel	85	90	110	140
Fish	120	135	160	180
Prawn	73	85	97	120
Squid	45	67	80	85
Crab	80	90	105	135
Squilla	120	125	168	195
b. *M.dobsoni*				
Oyster	95	110	130	165
Clam	125	140	159	182
Mussel	105	120	145	170
Fish	140	150	165	195
Prawn	45	45	60	75
Squid	48	50	75	105
Crab	78	90	100	115
Squilla	168	178	197	215

FIG 10 : Percentage response of M.dobsoni towards different extract fractions at 1% (W/v) concentration.

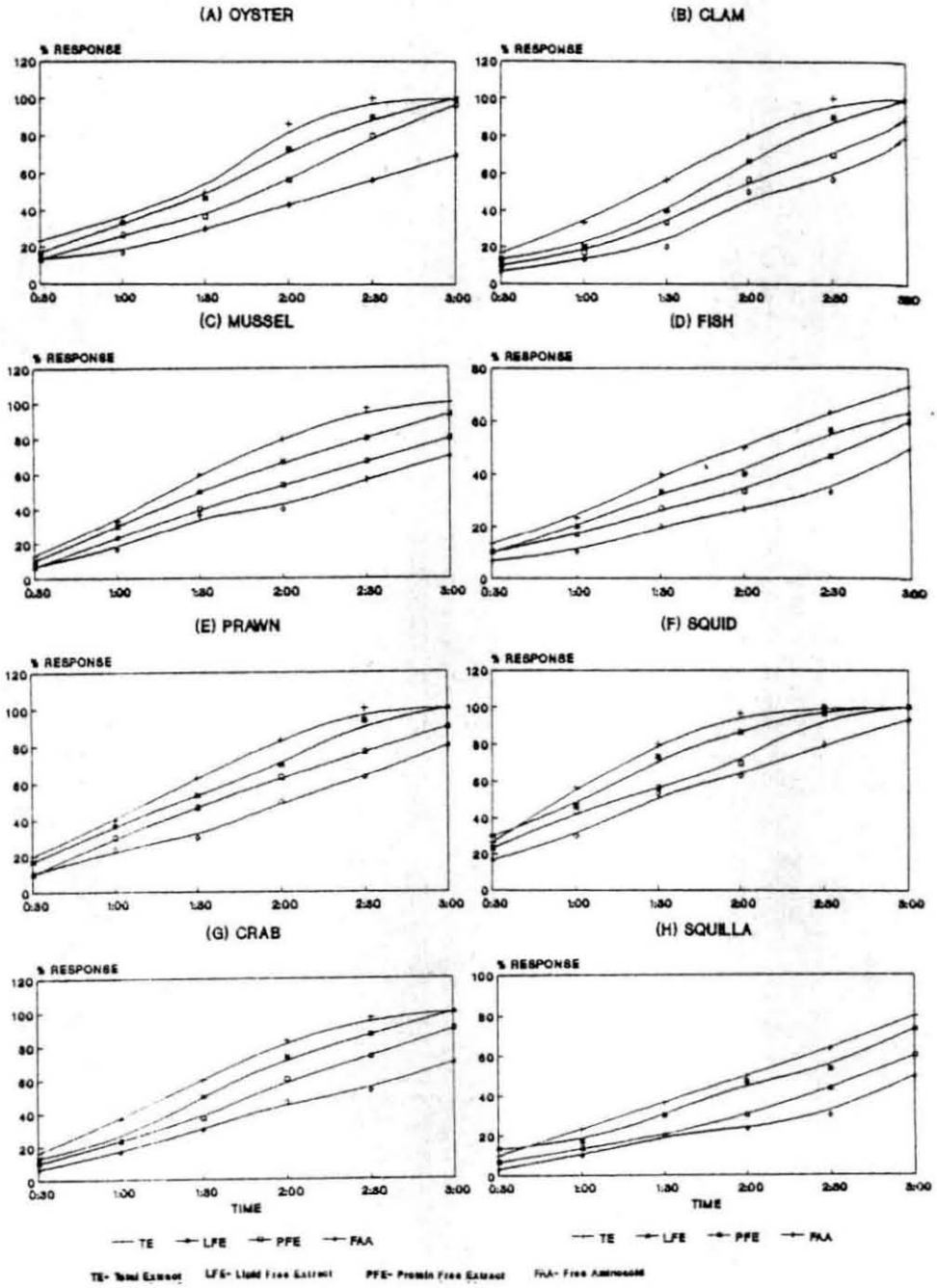


FIG 11 : Percentage response of P.indicus towards different extract fractions at 1% (W/V) concentration.

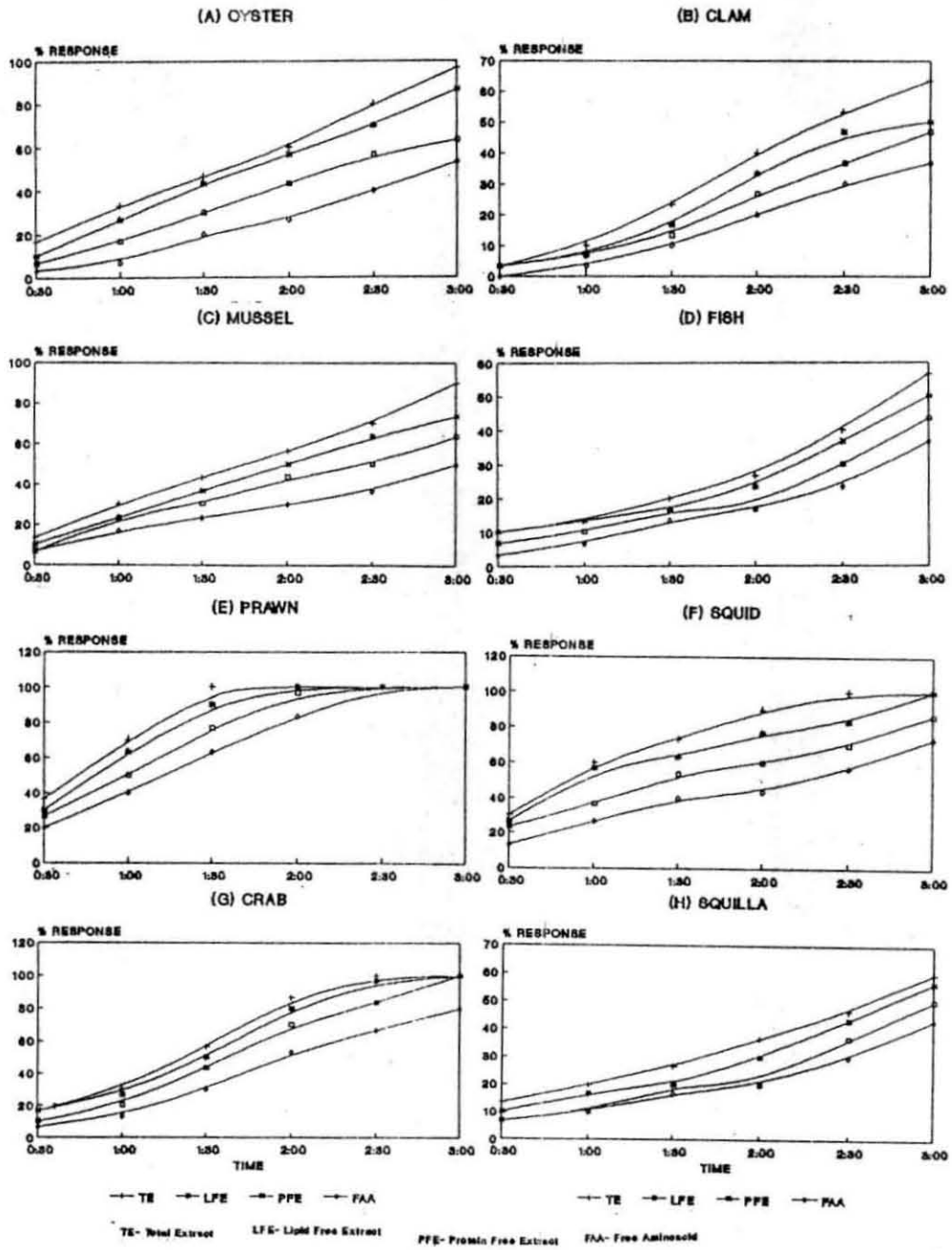


TABLE 19 : TIME TAKEN BY THE TEST ANIMALS (ET_{50}) TO LOCATE THE SOURCE OF STIMULUS.

TISSUE SOURCE	TOTAL EXTRACT	PROTEIN FREE EXTRACT	LIPID FREE EXTRACT	FREE AMINO ACID FRACTION
a. P.indicus				
Oyster	98.17	86.814	103.56	137.57
Black clam	79.70	95.95	13.82	135.24
Mussel	82.30	95.92	115.23	134.18
Fish	119.79	140.0	158.92	196.72
Prawn	73.26	83.59	98.32	123.12
Squid	55.69	60.98	78.29	86.67
Crab	77.40	90.964	109.20	137.05
Squilla	120.12	138.964	181.71	237.25
b. M.dobsoni				
Oyster	94.47	107.13	139.93	178.49
Black clam	146.93	172.98	196.0	233.34
Mussel	103.89	121.76	143.60	189.29
Fish	177.79	199.50	240.22	263.50
Prawn	42.09	48.59	59.14	72.67
Squid	53.50	54.66	93.41	130.82
Crab	79.30	84.38	97.05	120.08
Squilla	166.68	187.254	224.60	266.30

TABLE 20 : POTENCY OF DIFFERENT TISSUE EXTRACT FRACTIONS ON THE FEEDING BEHAVIOUR OF *PENAEUS INDICUS* AND *METAPENAEUS DOBSONI*

TISSUE TYPE	PROTEIN FREE EXTRACT		LIPID FREE EXTRACT		FREE AMINO ACID FRACTION	
	P.indicus	*M.dobsoni*	*P.indicus*	*M.dobsoni*	*P.indicus*	*M.dobsoni*
Oyster	0.772	0.761	0.938	0.962	0.495	0.436
Mussel	0.922	0.857	0.979	0.951	0.778	0.588
Fish	0.916	0.846	0.956	0.876	0.775	0.528
Clam	0.847	0.922	0.954	0.967	0.821	0.597
Prawn	0.904	0.707	0.994	0.899	0.888	0.396
Squid	0.819	0.882	0.983	0.940	0.773	0.444
Crab	0.859	0.860	0.979	0.978	0.847	0.389
Squilla	0.937	0.790	0.980	0.887	0.876	0.644

0.922 for M.dobsoni. In the case of extracts free from lipid it ranged between 0.938 and 0.994 for P.indicus and between 0.876 and 0.978 in M.dobsoni. The potency of Free Amino Acid (FAA) alone is between 0.495 and 0.888 in P.indicus and between 0.389 & 0.644 in M.dobsoni. The potencies of the test samples differed between the species also.

1.2.6. **Percentage Activity:** The percentage activity of different extract types is given in Table 21. The percentage activity decreases significantly ($P<0.01$) with the removal of different components from the extract. When the lipid fraction is removed from the extract the activity decreased and it ranged between 93.85% and 99.37% in P.indicus and 87.61% and 97.80% in M.dobsoni. When the free amino acid fraction alone was used it was between 49.59% and 88.78% and 38.91% and 64.35% for P.indicus and M.dobsoni respectively.

These findings indicate that the protein fraction contributes 12.8% of the chemotactic property of tissue extracts in P.indicus and 17.29% in M.dobsoni and the lipid fraction 3% and 6.75% respectively. The contribution of chemotactic activity by FAA fraction alone is 78.2% in P.indicus and 50.28% in M.dobsoni.

Among the extract types tested, the whole extract is the most effective feeding attractant and among tissue types squid followed by crab and oyster proved effective for P.indicus and in M.dobsoni it was shrimp followed by squid and crab.

Both P.indicus and M.dobsoni significantly differed in their responses to different tissue types tested ($P<0.05$). They showed a similar pattern of response to the extract types studied, but they differed significantly ($P<0.05$) in the degree of responses to different tissue components. Both the

TABLE 21 : PERCENTAGE ACTIVITY OF DIFFERENT TISSUE EXTRACT FRACTIONS ON THE FEEDING SBEHAVIOUR OF PENAEUS INDICUS AND METAPENAEUS DOBSONI

TISSUE TYPE	PROTEIN FREE EXTRACT		LIPID FREE EXTRACT		FREE AMINO ACID FRACTION	
	P.indicus (%)	M.dobsoni (%)	P.indicus (%)	M.dobsoni (%)	P.indicus (%)	M.dobsoni (%)
Oyster	77.22	76.12	93.85	96.23	49.59	43.59
Mussel	92.23	85.17	97.94	95.06	77.87	58.82
Fish	91.61	84.62	95.62	87.61	77.51	52.80
Clam	84.69	92.23	95.40	96.74	82.17	59.73
Prawn	90.43	70.66	99.37	89.92	88.78	39.62
Squid	81.89	88.25	98.30	94.02	77.27	44.39
Crab	85.92	85.97	97.91	97.80	84.72	38.91
Squilla	93.67	79.01	98.01	88.74	87.57	64.35

species differed in their feeding responses, EC_{50} values, potency and percentage activity. P.indicus was more receptive to the free amino acid fraction than M.dobsoni where as M.dobsoni is more receptive to protein and lipid fraction than P.indicus.

1.2.7. **Quantitative Response:** The behavioral responses of the shrimps are represented in Fig. 12 and 13 for P.indicus and M.dobsoni respectively and the average unit activity recorded in Table 22. The activity elicited by different test samples differed significantly ($P<0.01$) in their magnitude in both species. Among the extract types, whole extracts of all tissue sources produced more intense and strong feeding activity whereas it was less intense in the case of the free amino acid fraction. P.indicus produced maximum response to squid extracts and M.dobsoni to shrimp extract. Both species differ in their sensitivity to different test stimuli.

1.2.8. **Behaviour of Shrimps to the Stimuli:** P.indicus and M.dobsoni expressed grooming and feeding behaviour to the test sample. But the intensity and duration of the activity varied widely with the test samples. The behavioral indicators such as antennuler flicking movements, searching activity with pereopods, swimming movements of pleopods etc. are high for total extract, and least for the free amino acid fraction. Species differed in their response to different test stimuli. P.indicus produced intense food searching and feeding activity towards squid extract, while M.dobsoni responded more actively towards shrimp extract.

FIG 12 : Behavioural activity of P.indicus towards different extract fractions from different tissue sources recorded using Fish Activity Recorder.

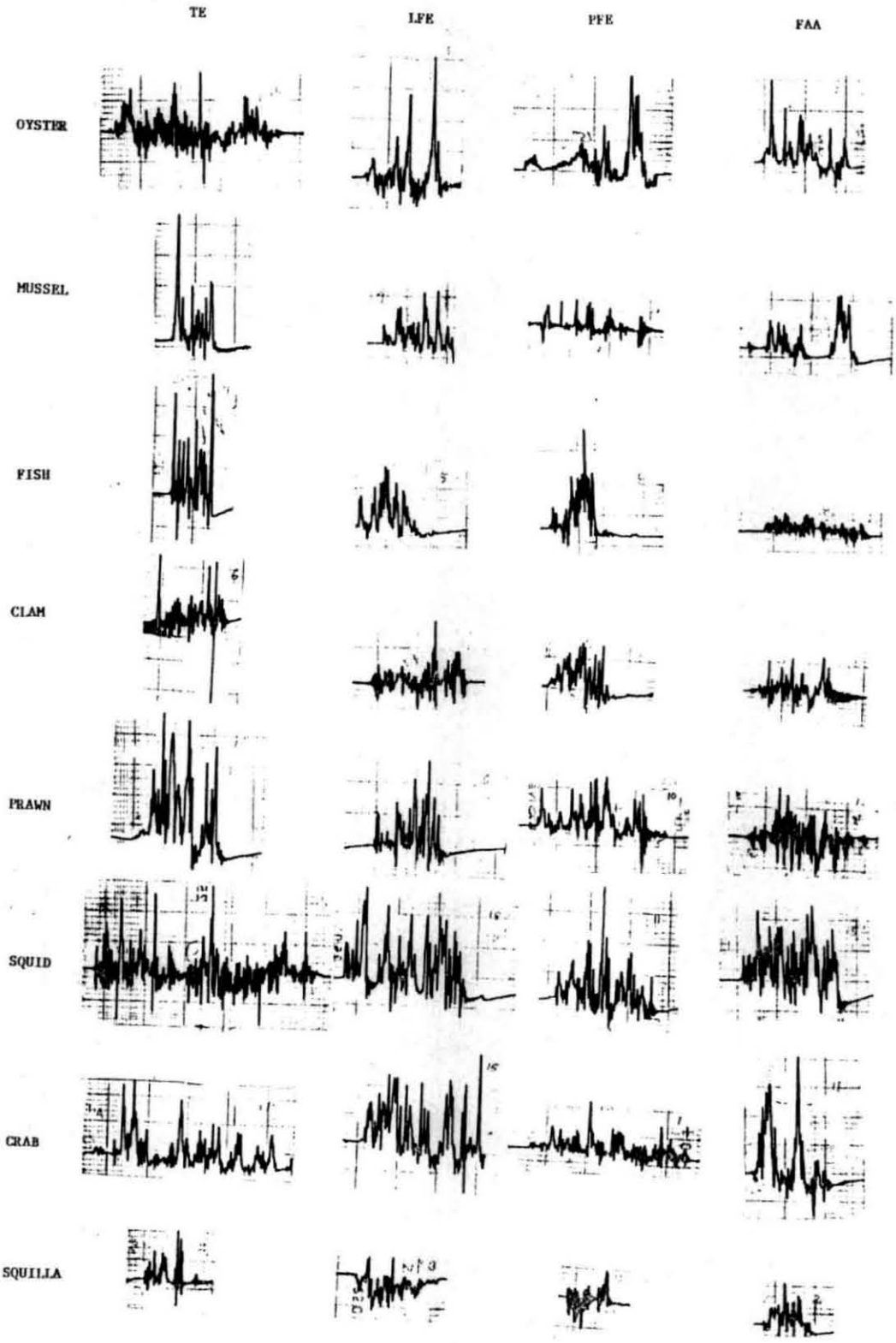


FIG 13 : Behavioural activity of M.dobsoni towards different extract fractions from different tissue sources recorded using Fish Activity Recorder.

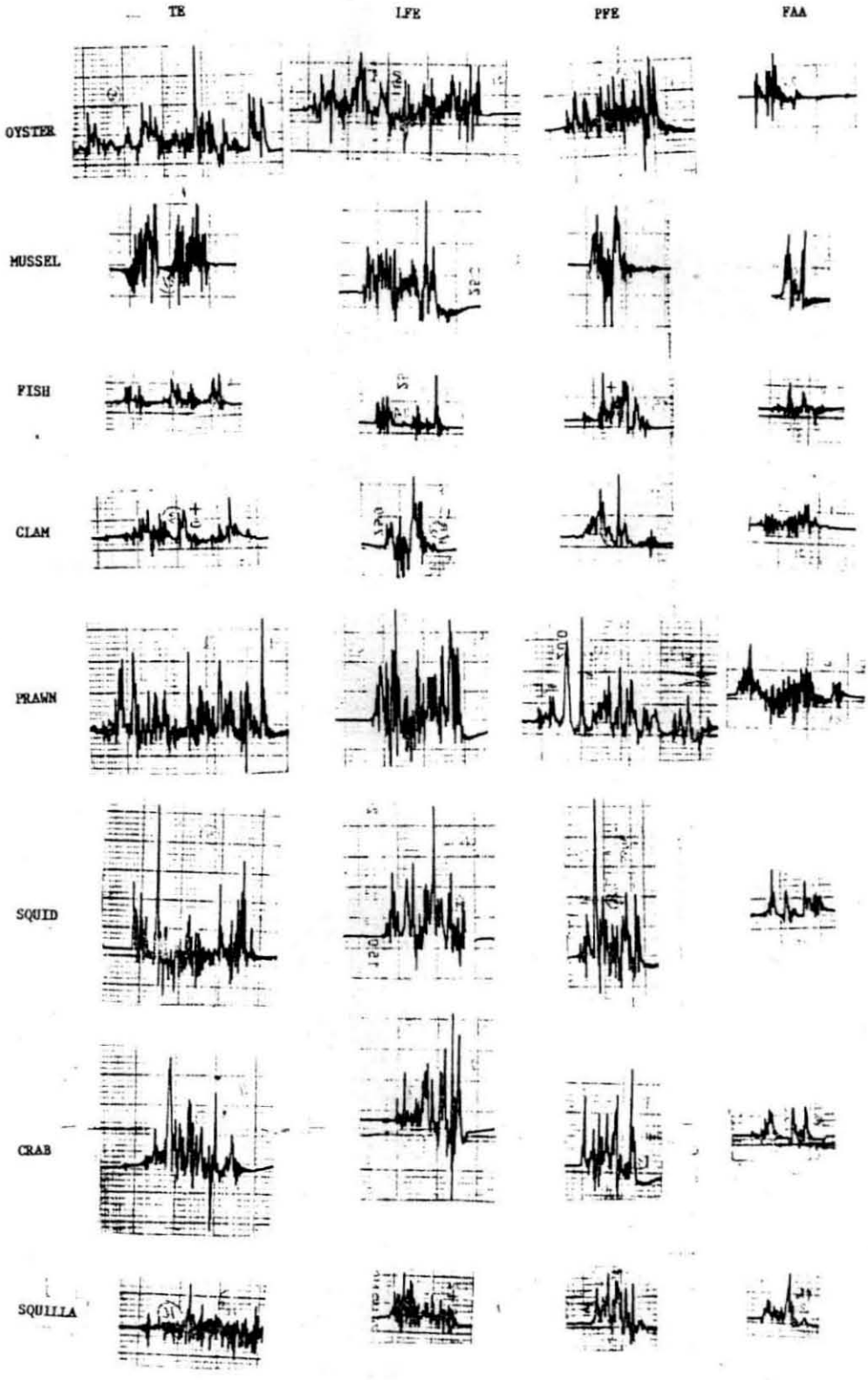


TABLE 22 : ACTIVITY RECORDED USING FISH ACTIVITY RECORDER FOR PENAEUS INDICUS AND METAPENAEUS DOBSONI TOWARDS DIFFERENT TEST STIMULUS (2% W/V)

TISSUE SOURCE	TOTAL EXTRACT	LFE	PFE	FAA
P.indicus				
Oyster	15	5	6	3
Mussel	5	4	5	4
Fish	7	5	6	2
Clam	6	6	4	3
Prawn	15	6	10	10
Squid	21	18	11	13
Crab	17	16	7	11
Squilla	2	3	2	2
M.dobsoni				
Oyster	13	12	10	2
Mussel	8	7	5	2
Fish	4	3	3	2
Clam	6	4	4	1
Prawn	18	10	12	3
Squid	13	10	8	2
Crab	11	8	7	2
Squilla	5	3	3	2

2. CHEMOTACTIC PROPERTY OF SYNTHETIC CHEMICAL STIMULI

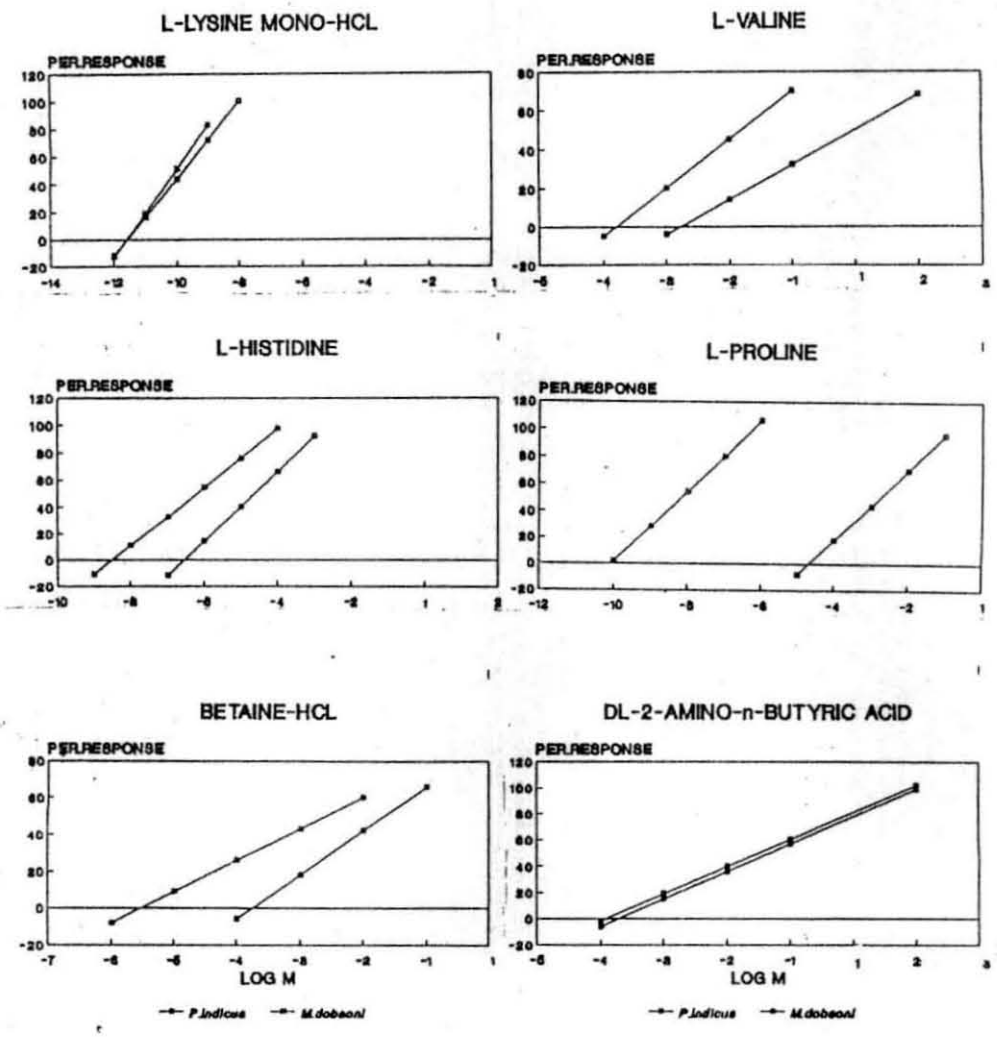
2.1. THRESHOLD CONCENTRATION

The percentage responses of sub-adult shrimps to different L-and DL - forms of amino acids are represented in Figs. 14a and 14b and the corresponding threshold concentration in Table 23. The intensity of activity recorded is represented in Fig (15a-c). The sensitivity of the shrimps towards different amino acids varies significantly ($P<0.05$). Animals were highly sensitive to amino acids like L-lysine, L-glycine, L-proline, L-alanine, L-phenylalanine, L-tryptophan, ornithine, L-methionine, L-isoleucine and taurine and were detected by both species at very low concentrations, whereas amino acids like L-cysteine, L-valine, DL-2-amino-n-butyric acid and DL-aspartic acid were detected at only higher concentrations. Species varied significantly in their sensitivity to various amino acids ($P<0.05$). In all cases P.indicus is more sensitive to amino acids than M.dobsoni except for L-phenylalanine which M.dobsoni detected at low concentration. Similarly both species were more sensitive to L-forms of amino acids than the DL-forms (Fig. 16). For eliciting the same amount of response, DL-amino acids required higher concentrations than L-forms. The activity recorded by the activity recorder also showed similar response.

2.2 BEHAVIOURAL RESPONSES OF SHRIMPS

When the amino acids were injected into the flow through bioassay system through the gauze the shrimps required higher concentrations to elicit feeding response (Table 24) than the TC_{50} stated above (Tables 23). Post-larvae, juveniles and sub-adults of both species differed significantly in their sensitivity to amino acids and related compounds ($P<0.05$). Juveniles followed

FIG 14 : Percentage response of P.indicus and M.dobsoni towards varying concentrations of amino acids.



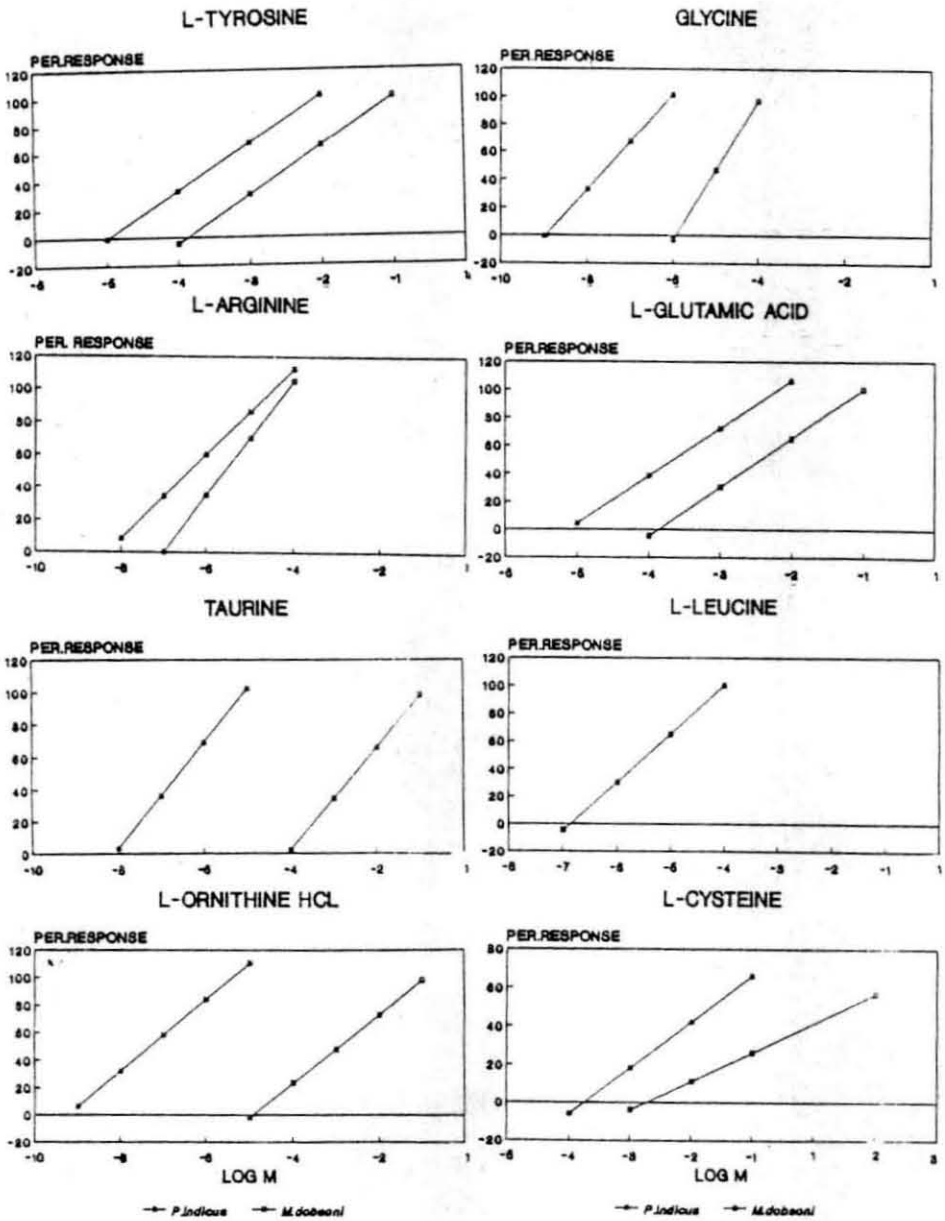

L-TYROSINE
GLYCINE
L-ARGININE
L-GLUTAMIC ACID
TAURINE
L-LEUCINE
L-ORNITHINE HCL
L-CYSTEINE
PER.RESPONSE
LOG M
P.indicus
M.dobsoni

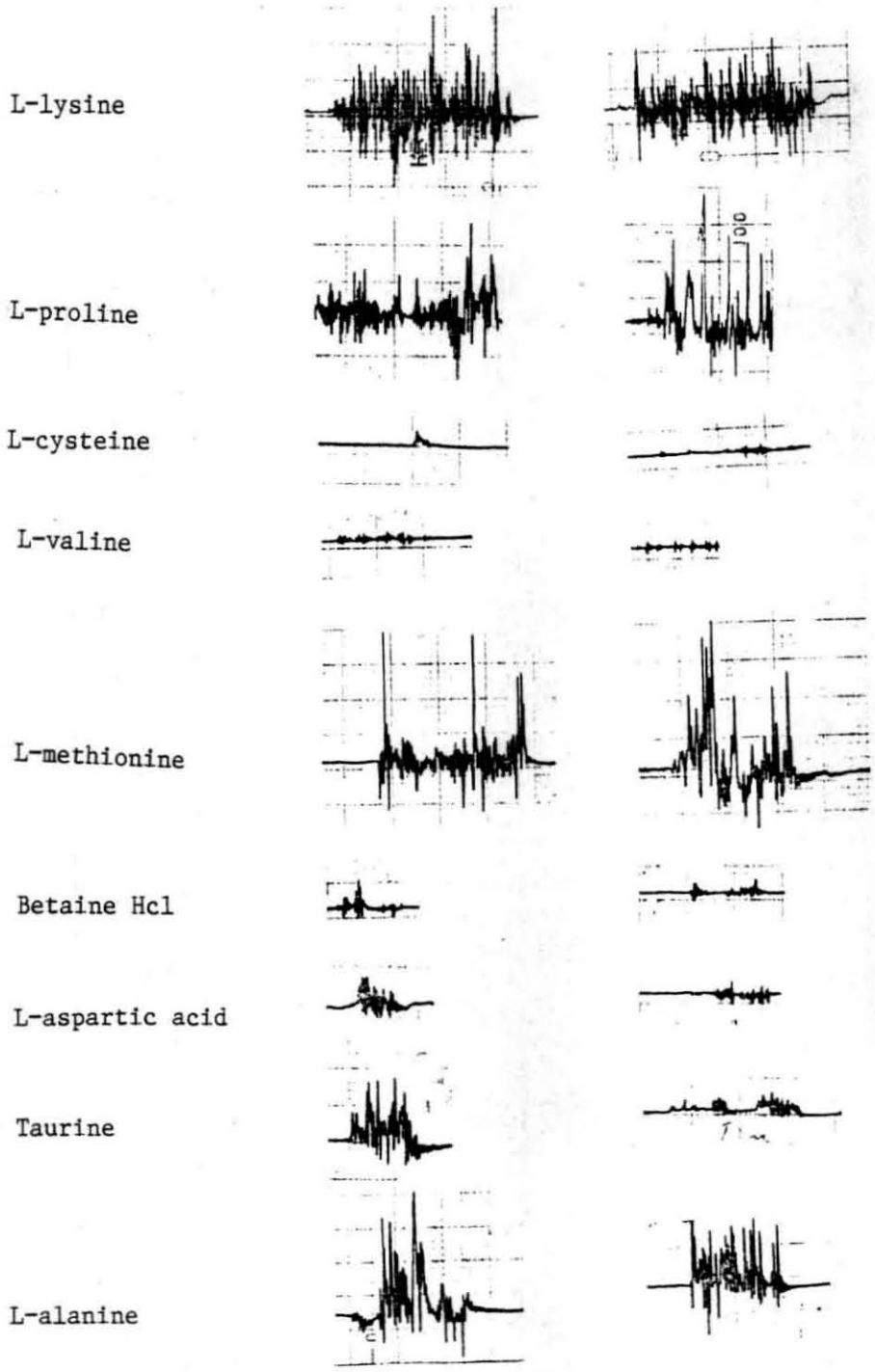
TABLE 23 : THRESHOLD CONCENTRATION OF AMINO ACIDS FOR SUB-ADULTS OF P.INDICUS AND M.DOBSONI

TEST STIMULI	P.indicus	M.dobsoni
L-glutamic acid	3.5×10^{-4}	5.5×10^{-3}
Glycine	4.5×10^{-8}	1.5×10^{-5}
L-arginine	5.6×10^{-7}	4.5×10^{-6}
L-leucine	6×10^{-6}	6×10^{-6}
L-tyrosine	5.5×10^{-4}	6×10^{-2}
Taurine	3×10^{-7}	4×20^{-3}
Ornithine	8×10^{-8}	1.5×10^{-3}
L-cysteine	4×10^{-2}	1.65
L-lysine	10^{-10}	2.5×10^{-10}
L-valine	2×10^{-2}	1.0
L-histidine	9×10^{-10}	5×10^{-5}
L-proline	8.5×10^{-9}	3.5×10^{-3}
Betaine HCl	$4x\ 10^{-3}$	3.5×10^{-2}
DL-2-amino-n-butyric acid	6.5×10^{-2}	4.5×10^{-2}
L-serine	4×10^{-4}	5.5×10^{-2}
DL-serine	3×10^{-3}	3×10^{-3}
L-alanine	4.5×10^{-7}	8.5×10^{-8}
DL-alanine	5×10^{-4}	6.5×10^{-3}
L-phenylalanine	9×10^{-8}	3×10^{-9}
DL-phenylalanine	4.5×10^{-4}	6×10^{-4}

Table 23 (Contd..)

TEST STIMULI	P.indicus	M.dobsoni
L-aspartic acid	3.5×10^{-4}	8.5×10^{-4}
DL-aspartic acid	4×10^{-2}	8.5×10^{-2}
L-tryptophan	8.5×10^{-7}	6.0×10^{-6}
DL-tryptophan	6.5×10^{-5}	8×10^{-5}
L-threonine	2×10^{-5}	4×10^{-5}
DL-threonine	10^{-4}	3.5×10^{-3}
L-isoleucine	10^{-6}	1.5×10^{-5}
DL-isoleucine	7.0×10^{-4}	3.5×10^{-3}
L-methionine	0.5×10^{-9}	1.5×10^{-8}
DL-methionine	5.5×10^{-5}	7.5×10^{-4}
Sucrose	5×10^{-3}	2×10^{-2}
Glucose	9.5×10^{-4}	1.5×10^{-3}

FIG 15 : Behavioural activity of P.indicus and M.dobsoni towards different amino acids at 10^{-1}M concentration.



L-threonine

L-serine

L-tyrosine

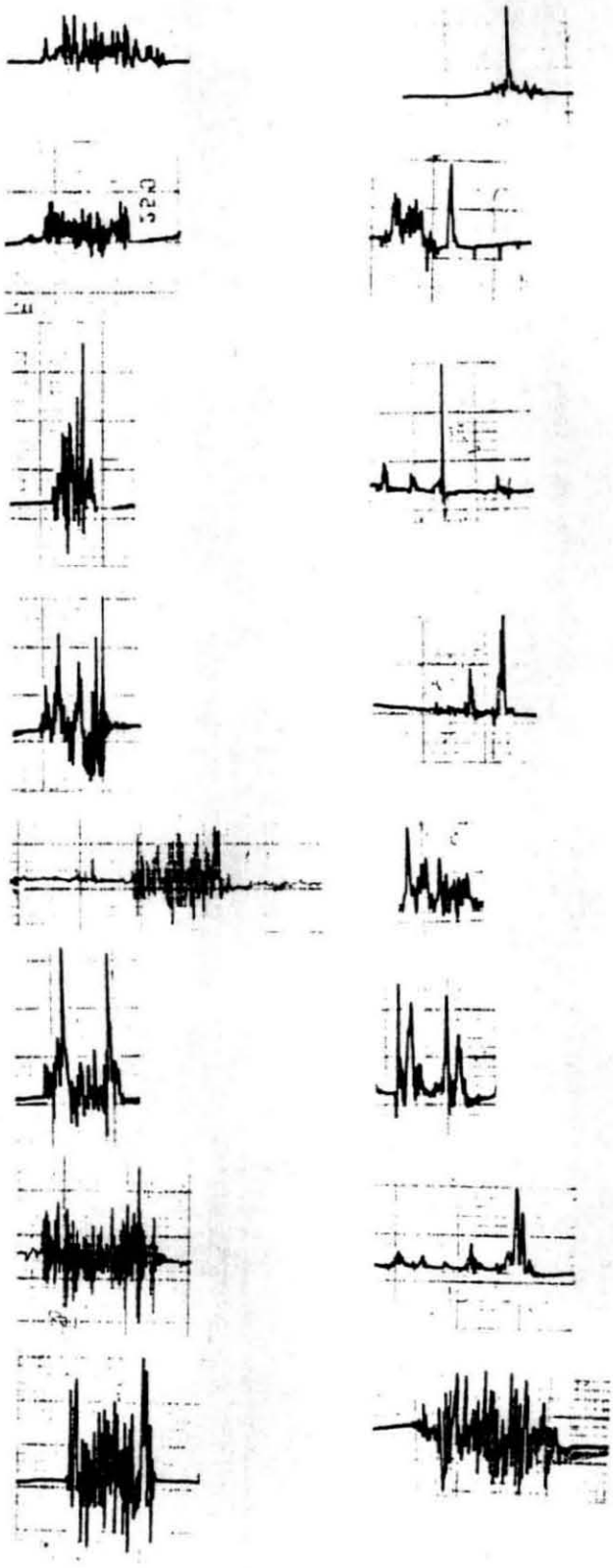
L-glutamic acid

L-histidine

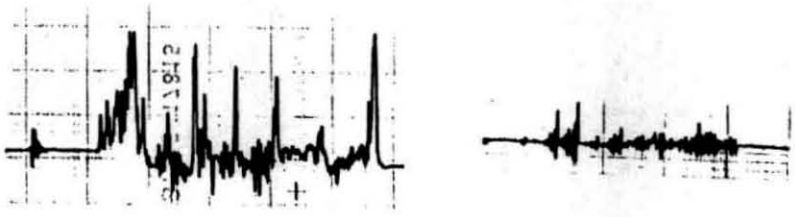
L-leucine

L-isoleucine

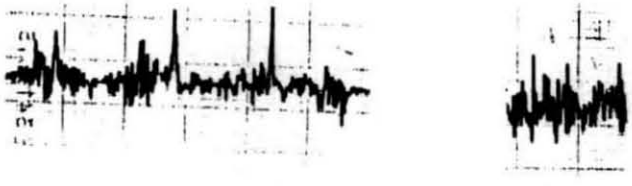
L-phenylalanine



Glycine



L-tryptophan



L-arginine

L-ornithine

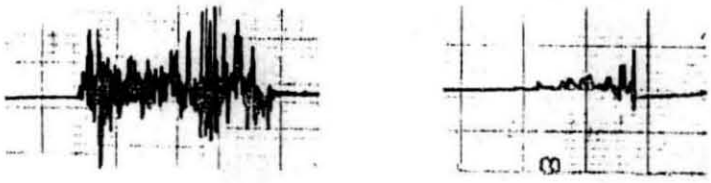


FIG 16 : Percentage response of P.indicus and M.dobsoni towards L-forms and DL-forms of amino acids at different concentrations.

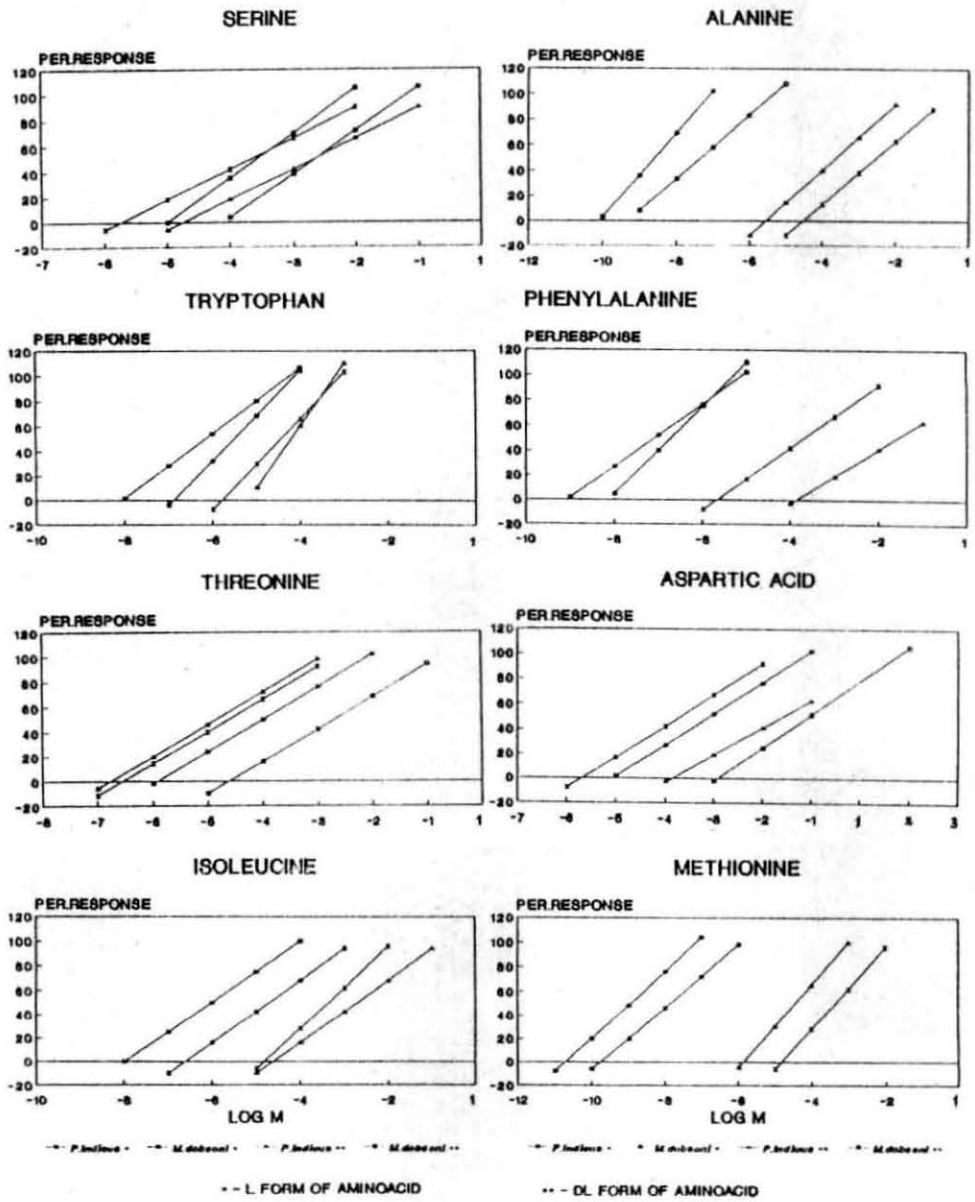


TABLE 24 : THE LOWEST CONCENTRATION DETECTED BY UNIFORM STAGES OF *P.INDICUS* & *M.DOBSONI* GIVEN IN THE FLOW THROUGH BIOASSAY SYSTEM

AMINO ACID	*P.indicus*			*M.dobsoni*		
	PL	JUVENILE	SUBADULT	PL	JUVENILE	SUBADULT
L-alanine	10^{-2}	10^{-1}	10^{-5}	1.5×10^{-1}	1.25	2.5×10^{-4}
Taurine	1.25×10^{-2}	8.5×10^{-2}	3×10^{-3}	10^{-1}	1.4	2×10^{-3}
Arginine	6×10^{-3}	7.0×10^{-2}	7.0×10^{-3}	9×10^{-3}	7.5×10^{-2}	1.5×10^{-2}
Tryptophan	1.5×10^{-3}	4×10^{-5}	9.5×10^{-3}	1.5×10^{-2}	6.0×10^{-2}	4.0×10^{-2}
Lysine	8×10^{-4}	9×10^{-6}	2.5×10^{-5}	10^{-3}	7.0×10^{-6}	10^{-4}
Methionine	8×10^{-4}	9×10^{-6}	9×10^{-6}	1.5×10^{-2}	9×10^{-5}	10^{-3}
Glycine	7.5×10^{-2}	5.5×10^{-4}	3×10^{-3}	10^{-1}	8.5×10^{-5}	1.5×10^{-2}
Serine	1.6	2.0×10^{-3}	2×10^{-2}	1.1	7.5×10^{-1}	2.0×10^{-1}

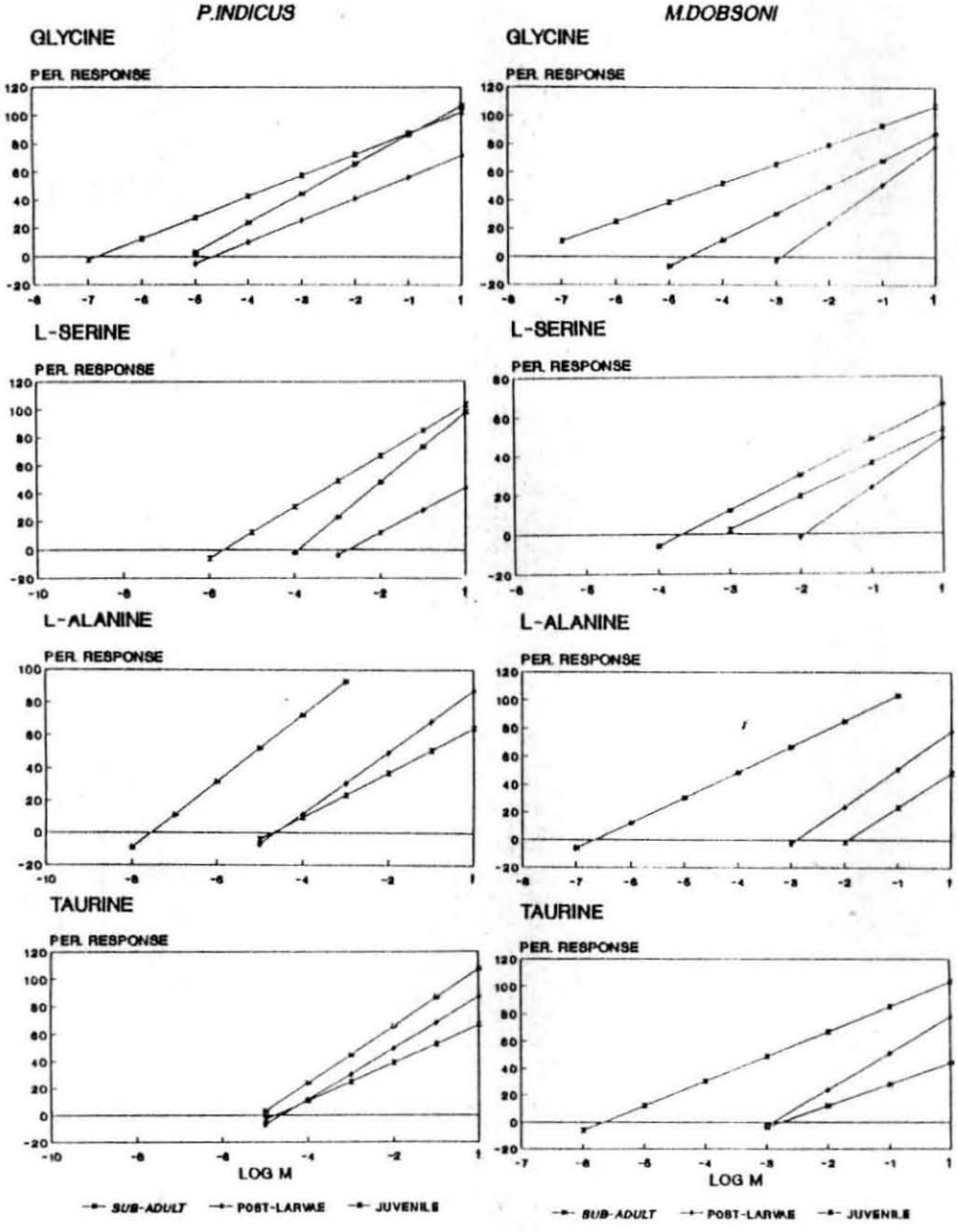
by sub-adults are more sensitive towards the stimulus at lower concentrations (Fig. 17a & b). Post-larvae detected all stimuli only at a high concentration and they were seen in clusters at the stimulus source whereas the juveniles and sub-adults arrived one by one towards the source. As in the case of amino acids the shrimps differed in their sensitivity to different sugars. P.indicus was more sensitive than M.dobsoni and detected sugars at lower concentrations (Fig. 18 & Table 23).

Responses of animals to amino acids and sugars are given in Table 25. Response to same level of various test stimulus varied significantly for both species ($P<0.05$). Species also significantly differed in their response towards the same test stimuli. P.indicus was more responsive to all the stimuli than M.dobsoni. L-lysine, L-methionine, L-glycine, L-alanine, L-proline, L-phenylalanine, L-leucine and taurine produced higher responses in P.indicus, where as the order of preference for M.dobsoni was L-lysine, L-methionine, L-alanine, L-phenylalanine, L-leucine, L-glycine, L-tryptophan, and L-isoleucine. Betaine, L-valine and L-cysteine produced no response in M.dobsoni. L-cysteine in P.indicus produced no response, but betaine and L-valine produced very weak response. In both species the response elicited by sugars is also very low.

2.3. PROPERTY OF CHEMICAL STIMULI

Feeding property of test stimuli in both species is given in table 26. Based on the response of animals to these stimuli, they were classified as attractant, incitant and stimulant. Most of the test compounds produced only one of the above property. But some compounds act in different ways at different concentrations. Glycine, and lysine mono-HCl acts as an attractant, incitant and stimulant in the same animal at different concentrations. At low

Fig 17 : Percentage response of post-larvae, juveniles and sub-adults of P.indicus and M.dobsoni towards varying concentrations of amino acids.



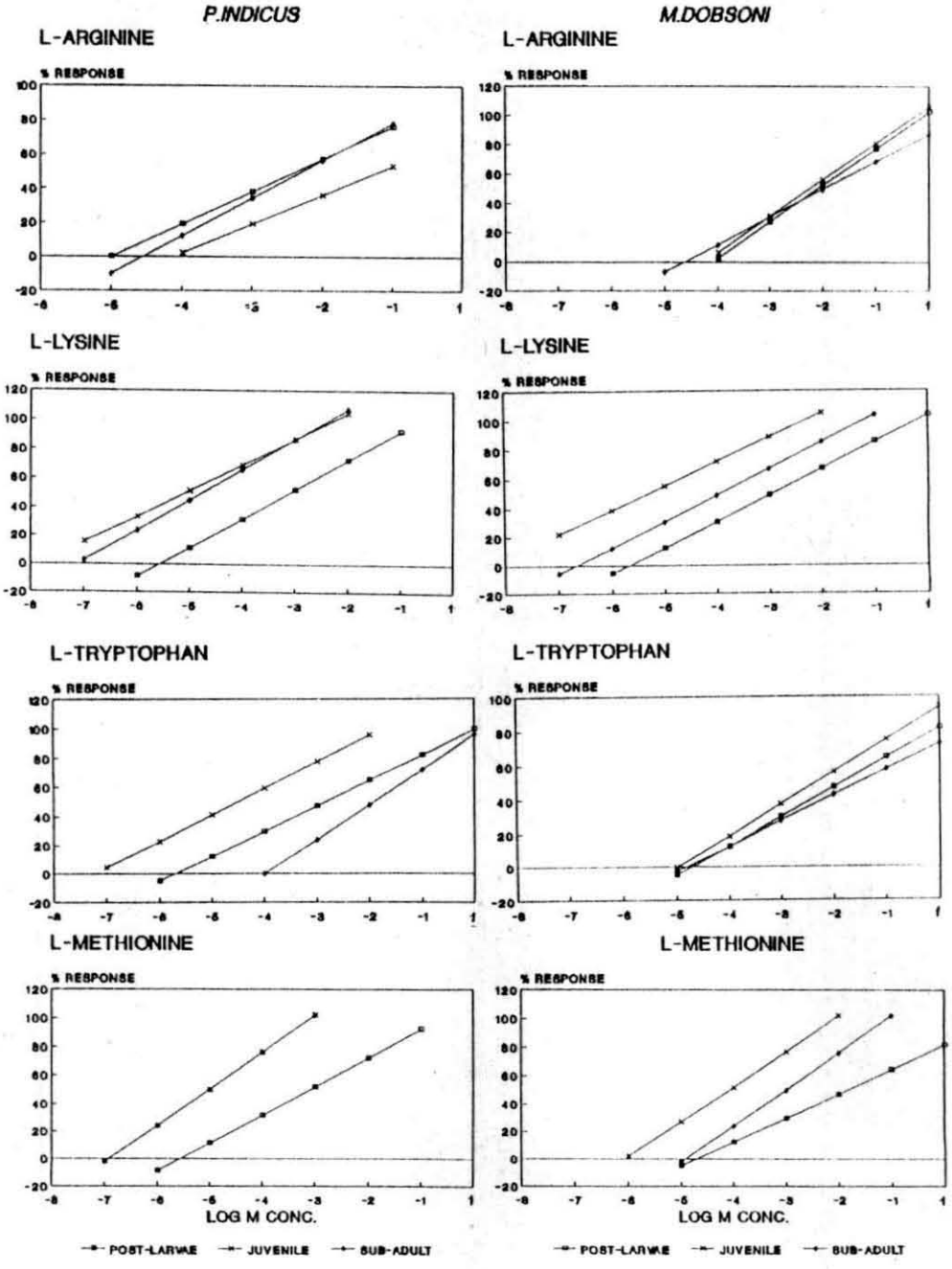
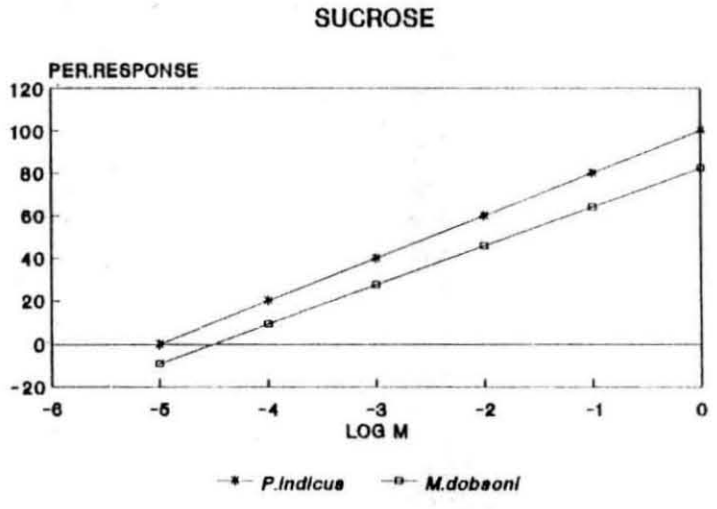

P.INDICUS
M.DOBSONI
L-ARGININE
L-ARGININE
% RESPONSE
% RESPONSE
L-LYSINE
L-LYSINE
% RESPONSE
% RESPONSE
L-TRYPTOPHAN
L-TRYPTOPHAN
% RESPONSE
% RESPONSE
L-METHIONINE
L-METHIONINE
% RESPONSE
% RESPONSE
LOG M CONC.
LOG M CONC.
POST-LARVAE
JUVENILE
SUB-ADULT
POST-LARVAE
JUVENILE
SUB-ADULT

FIG 18 : Percentage responses of P.indicus and M.dobsoni towards varying concentrations of sugars.



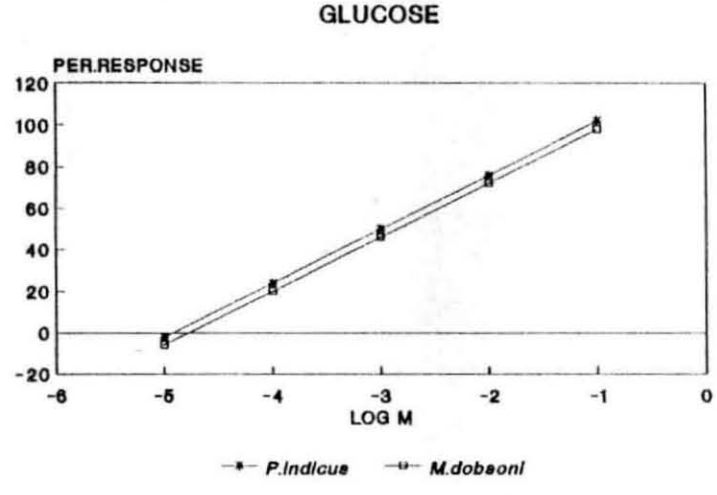


TABLE 25 : PERCENTAGE RESPONSE OF P.INDICUS AND M.DOBSONI JUVENILES TO CHEMICAL STIMULI OF (10^{-1}M) CONCENTRATION

TEST STIMULI	P.indicus	M.dobsoni
L-glutamic acid	50.33	56.67
Glycine	86.66	73.33
L-arginine	66.66	56.66
L-leucine	76.67	76.67
L-tyrosine	33.33	20.22
Taurine	70.12	35.56
Ornithine	50.66	46.67
L-cysteine	No response	No response
L-lysine	96.67	93.33
L-valine	3.26	No response
L-histidine	67.66	52.26
L-proline	33.33	43.36
Betaine HCl	4.66	No response
DL-2-amino-n-butyric acid	23.32	16.67
L-serine	26.67	28.13
L-alanine	83.66	79.76
L-phenylalanine	80.12	77.76
L-aspartic acid	40.77	51.33
L-tryptophan	66.62	71.33
L-threonine	50.17	45.67
L-isoleucine	69.13	64.33
L-methionine	92.66	91.23
Sucrose	12.32	10.12
Glucose	13.412	9.667

TABLE 26 : FEEDING PROPERTY OF CHEMICAL FEEDING EFFECTORS

L-glutamic acid	Incitant (1M)
Glycine	Attractant (10^{-1}M)
	Incitant (10^{-1}M)
	Stimulant (1M)
L-serine	Incitant (10^{-3}M)
L-alanine	Stimulant (10^{-5}M)
L-phenylalanine	Attractant (10^{-5}M)
L-arginine	Incitant (10^{-1}M)
L-leucine	Incitant (1M)
Taurine	Stimulant (10^{-3}M)
L-aspartic acid	Incitant (1M)
L-tryptophan	Stimulatn (1M)
L-proline	Incitant (10^{-7}M)
	Stimulant (10^{-5}M)
L-ornithine HCl	Incitant (10^{-5}M)
L-cysteine	Arrestant
L-lysine mono-HCl	Attractant (10^{-9}M)
	Incitant (10^{-9}M)
	Stimulant (10^{-6}M)
L-valine	Arrestant
L-isoleucine	Incitant
L-histidine	Attractant (10^{-9}M)
L-methionine	Incitant (10^{-5}M)
Betaine HCl	Arrestant (1M)

concentration, above the threshold concentration they act as an attractant, and at a still higher concentration as an incitant. But at much higher concentration they acted as a feeding stimulant. Amino acids like tyrosine, threonine and isoleucine and sugars, though elicited some feeding behavior in shrimps, have no role as an attractant, incitant or as a stimulant. Whereas betaine - HCL, L-valine, and L-cysteine, when injected into the system arrested the movement of animals near the source of stimuli.

3. EFFECT OF WATER QUALITY PARAMETERS ON CHEMORECEPTION

3.1. EFFECT OF SALINITY ON FEEDING RESPONSE

3.1.1. **Behavioural Response:** Behaviour of P.indicus and M.dobsoni varied qualitatively and quantitatively with salinity. Animals elicited typical feeding behaviours at all the salinity levels tested. But its intensity varied with salinity change. In P.indicus, food searching and grooming activities were higher at 20%. salinity followed by 25%. and minimum at 5 and 35%.; whereas in M.dobsoni, maximum activity was observed at 15%. salinity followed by 20%. and minimum at 35%..

3.1.2. **Time Lag to Respond:** The time lag to elicit various feeding behaviours at different salinity levels are presented in Tables 27 and 28 which indicated that salinity has significant influence ($P<0.05$) on the latency period to respond. The time lag was minimum at 20%. salinity for post-larvae, at 25%. for juveniles and sub-adults of P.indicus and at 20%. for post-larvae and 15%. for juveniles and sub adults of M.dobsoni. The maximum time lag in P.indicus post -larvae and sub-adult was at 5%. and juveniles at 35%. and in M.dobsoni it was at 35%. for all the three stages of animals.

TABLE 27 : TIME LAG IN SECONDS TO ELICIT FEEDING RESPONSES IN PENAEUS INDICUS UNDER VARYING SALINITY.

Feeding Behaviour	Salinity %. 5	10	15	20	25	30	35
(a) Post-larvae							
Perception	123.50	96.00	62.00	51.00	61.00	77.00	90.00
Displacement	130.00	121.00	72.00	64.00	71.00	86.00	110.00
Arrival	161.00	136.00	95.00	80.00	84.00	99.00	122.00
Ingestion	172.00	140.00	113.00	84.00	91.00	118.00	144.00
(b) Juveniles							
Perception	79.00	52.00	39.00	38.00	31.00	55.00	81.00
Displacement	87.00	84.00	53.00	46.00	38.00	72.00	92.00
Arrival	112.00	103.00	71.00	66.00	57.00	91.00	121.00
Ingestion	119.00	112.00	80.00	73.00	68.00	102.00	129.00
(c) Sub adult							
Perception	116.00	92.00	51.00	41.00	32.00	65.00	82.00
Displacement	127.00	104.00	63.00	51.00	49.00	78.00	100.00
Arrival	149.00	124.00	92.00	76.00	66.00	95.00	127.00
Ingestion	160.00	137.00	106.00	82.00	79.00	105.00	130.00

TABLE 28 : TIME LAG (IN SECONDS) TO ELICIT FEEDING RESPONSE IN METAPENAEUS DOBSONI UNDER VARYING SALINITY LEVELS.

Feeding Behaviour	Salinity %. 5	10	15	20	25	30	35
(a) Post-larvae							
Perception	96.00	84.00	74.00	70.00	75.00	81.00	98.00
Displacement	107.00	92.00	89.00	83.00	91.00	103.00	125.00
Arrival	131.00	117.00	107.00	105.00	116.00	127.00	147.00
Ingestion	164.00	131.00	118.00	108.00	127.00	138.00	161.00
(b) Juveniles							
Perception	83.00	63.00	51.00	54.00	62.00	71.00	86.00
Displacement	96.00	72.00	54.00	61.00	69.00	84.00	100.00
Arrival	122.00	99.00	73.00	80.00	93.00	101.00	126.00
Ingestion	129.00	114.00	82.00	91.00	113.00	113.00	144.00
(c) Sub adult							
Perception	91.00	67.00	55.00	60.00	65.00	76.00	93.00
Displacement	98.00	78.00	64.00	68.00	77.00	93.00	111.00
Arrival	131.00	114.00	92.00	98.00	101.00	112.00	136.00
Ingestion	154.00	126.00	107.00	112.00	129.00	129.00	158.00

The time lag to locate the stimulus source and to initiate ingestion activity by 50% of the test animals (ET_{50} values) at different salinity levels are given in table 29. In P.indicus post-larvae the small ET_{50} value of 84.35 seconds was obtained at 20%. salinity and for juveniles (79.32 seconds) and sub-adults (83.7 seconds) at 25%. salinity. Large ET_{50} values were obtained at 5%. for all the three stages of test animals. In M.dobsoni small ET_{50} value for post-larvae (88.36) occurred at 20%. and for juveniles large values were obtained at 35%. for post-larvae and at 5%. for juveniles and sub-adults. The ET_{50} values varied significantly with the salinity of the rearing medium ($P<0.05$).

3.1.3. **Group Response** : Percentage response of test animals at different salinity levels are given in table 30. Salinity influenced the response of animals significantly ($P<0.05$). The response was higher at 20%. salinity for the post-larvae (83.33%) and juveniles (96.67%) and at 25%. for sub-adults (36.67) of P.indicus and it was minimum at 5%. salinity. In M.dobsoni maximum response occurred at 20%. for post-larvae (80%) and at 15%. for juveniles (86.67%) and sub-adults (86.67%) with minimum response at 5%. • Post-larvae, juveniles and sub-adults elicited the same pattern of activity at the test salinities with slight variation in their affinity to salinity levels. Among the various stages of animals tested juveniles were more responsive to chemotactic stimulus followed by sub-adults and least by post-larvae.

The behavioural intensity of the test animals to the test stimuli at various salinity levels are depicted in Fig. 19 and Table 31. In P.indicus intense activity was recorded at 25%. (7 units) and minimum at 5%. (2 units) and in M.dobsoni the same was at 15%. (8 units) and 5%. and 35%. (2 units) respectively.

TABLE 29 : TIME LAG TO LOCATE THE STIMULUS SOURCE BY 50% OF THE TEST ANIMALS.

	SALINITY %.						
	5	10	15	20	25	30	35
(A) P.indicus							
Post-larvae	99.54	97.33	85.80	84.35	86.74	87.68	96.50
Juveniles	95.16	91.71	81.22	81.49	79.32	85.23	90.84
Sub adults	99.27	92.72	86.78	84.26	83.70	86.85	93.50
(B) M.dobsoni							
Post-larvae	98.85	95.40	89.36	88.36	91.80	96.20	104.08
Juveniles	91.89	87.31	79.30	81.27	84.83	88.44	90.80
Sub adults	96.67	90.83	81.99	84.25	87.50	92.73	94.22

TABLE 30 : PERCENTAGE RESPONSE (LOCATION OF STIMULUS SOURCE) OF THE TEST ANIMALS DURING 2 MINUTE OBSERVATION.

	SALINITY %.						
	5	10	15	20	25	30	35
(A) P.indicus							
Post-larvae	60.0	66.67	80.0	83.33	80.00	76.67	66.67
Juveniles	70.0	70.0	86.67	96.67	90.00	83.33	76.67
Sub adults	66.67	70.0	83.33	83.33	86.67	83.33	73.33
(B) M.dobsoni							
Post-larvae	63.33	66.67	76.67	80.0	76.67	70.0	66.67
Juveniles	66.67	73.33	86.67	83.33	83.33	80.00	76.67
Sub adults	66.67	70.0	86.67	83.33	83.33	76.67	73.33

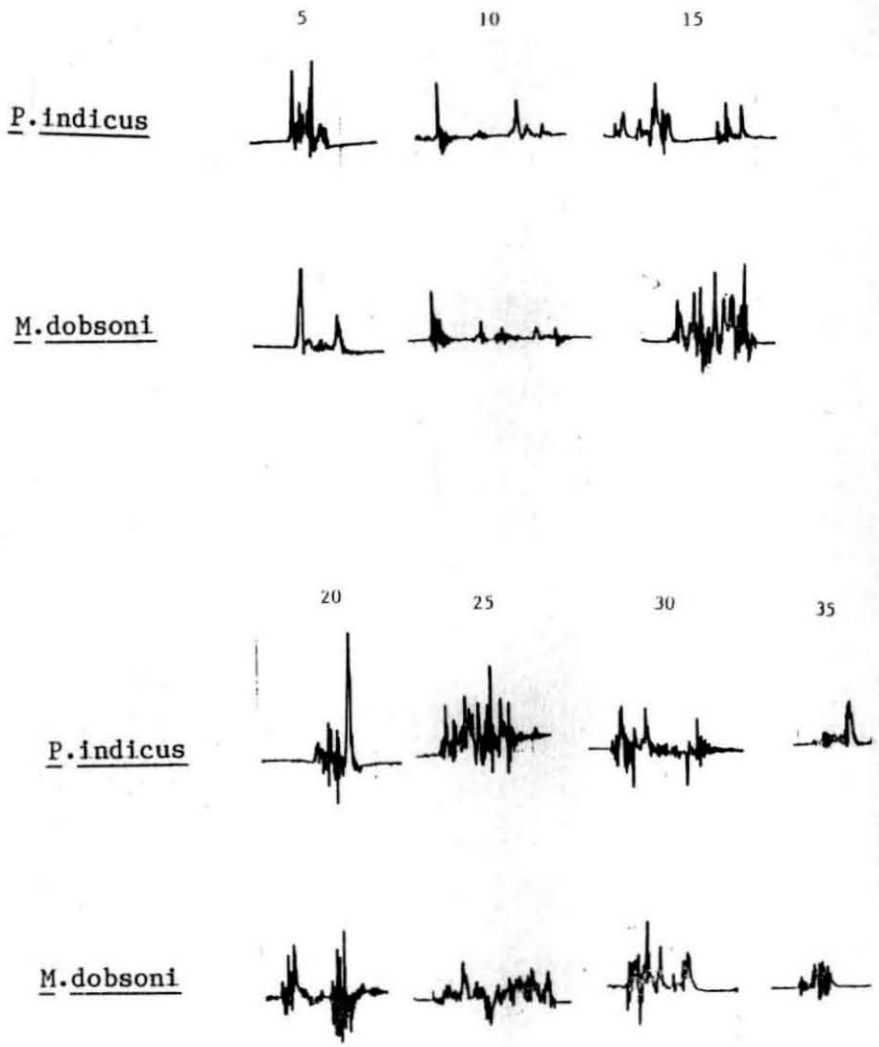
TABLE 31 : ACTIVITY (IN UNITS) OF THE TEST ANIMALS (SUB ADULTS) AT DIFFERENT SALINITY LEVELS.

Salinity (%.)	P.indicus	M.dobsoni
5	2	2
10	3	3
15	4	8
20	6	5
25	7	4
30	5	3
35	3	2

TABLE 32 : FEED CONSUMED BY THE TEST ANIMALS (G/100 G. BODY WEIGHT) AT DIFFERENT SALINITY LEVELS AFTER 2 HR. PERIOD.

	SALINITY %.						
	5	10	15	20	25	30	35
P.indicus							
Post-larvae	3.44	3.385	3.96	4.04	4.015	3.860	3.560
Juveniles	2.39	2.45	2.78	2.86	2.82	2.740	2.230
Sub adults	0.940	0.960	0.954	1.18	1.21	1.18	1.136
M.dobsoni							
Post-larvae	3.270	3.130	3.560	3.684	3.62	3.42	3.36
Juveniles	2.31	2.46	2.91	2.87	2.38	2.43	2.38
Sub adults	0.870	1.018	1.09	1.033	0.98	0.96	0.866

FIG 19 : Behavioural activity of P.indicus and M.dobsoni towards the test stimulus at varying levels of salinity.



3.1.4. **Feeding Response:** Feed intake of shrimps at different salinity levels are given in table 32. The feed intake by the test animals varied significantly with salinity ($P<0.05$). The post-larvae and juveniles of P.indicus showed maximum feed intake (4.04 and 2.86g per 100g body weight respectively) at 20%. and sub adults at 25%. salinity (1.21g.). The feed consumption was minimum at 5%. for post-larvae and sub-adults and at 35%. for juveniles. In M.dobsoni maximum fed intake was observed at 20%. for post-larvae (3.684 g.) and at 15%. for juveniles (2.87 g.) and sub-adults (1.033 g.) and minimum at 5%. for post-larvae and juveniles and at 35%. for sub-adults.

3.2. EFFECT OF pH ON FEEDING RESPONSE

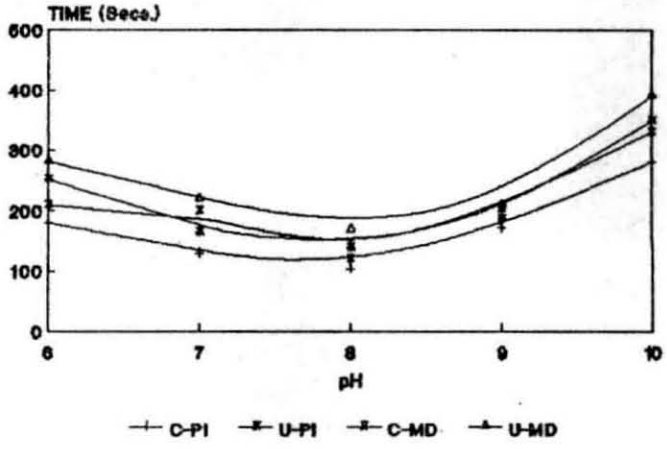
3.2.1. **Behavioural Response:** Behavioural responses of the shrimps to the feeding stimulus varied considerably with pH. Feeding behaviour was intense and more frequent at pH 8.0 and 9.0. Grooming did not vary considerably at pH between 7.0 and 9.0, but on lowering the pH to 6.0 or on increasing to 10.0 the grooming activity declined drastically. Searching movements were highest at pH 8.0 for all stages of test animals and minimum at pH 6.0 for juveniles and sub-adults and at 10.0 for post larvae. Waving action of the antennule varied with the pH, being maximum at pH 8.0 and minimum at pH 6.0 and 10.0. After detecting the presence of stimulus, the animals located the stimulus source by active swimming. But at pH 6.0 and 10.0 the orientation and swimming activity became very weak and declined considerably.

3.2.2. **Time Lag to Respond:** The latency to elicit feeding responses at various pH levels are represented in Fig. 20 (a-d). The time lag between the introduction of stimulus and the initiation of feeding response varied with pH of the rearing medium. The time lag was small at pH 8.0 for both species

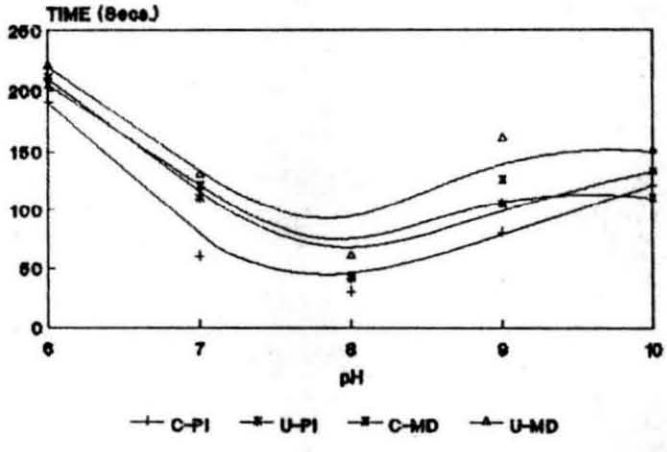
FIG 20 : Latency to elicit various feeding responses in post-larvae, juveniles and sub-adults of P.indicus and M.dobsoni towards stimulus sources at different pH levels.

PERCEPTION

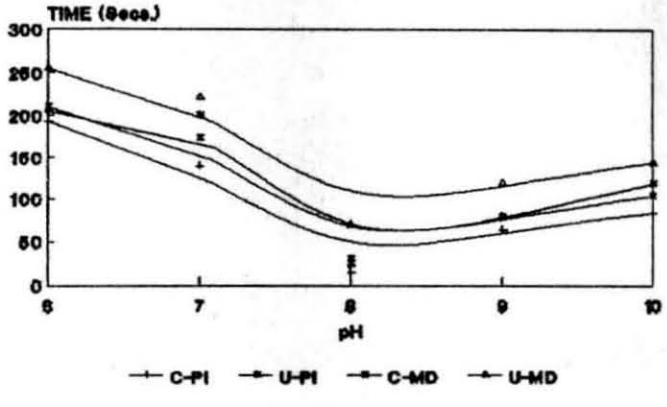
POST-LARVAE



JUVENILE



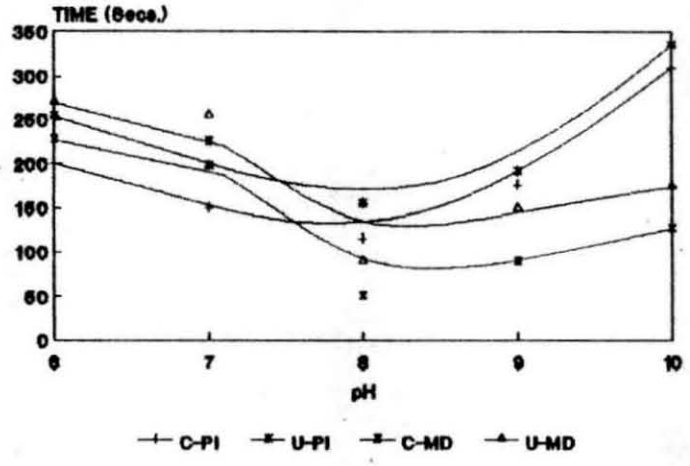
SUB-ADULT



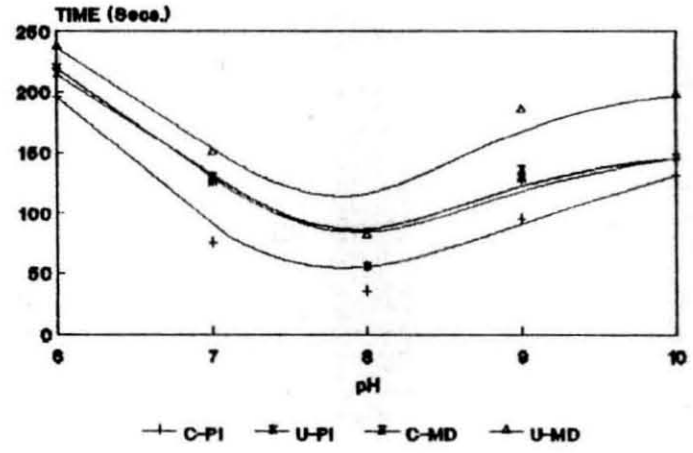
C- Coated feed, U- Uncoated feed
PI-*Penaeus indicus*, MD- *Metapenaeus dobsoni*

DISPLACEMENT

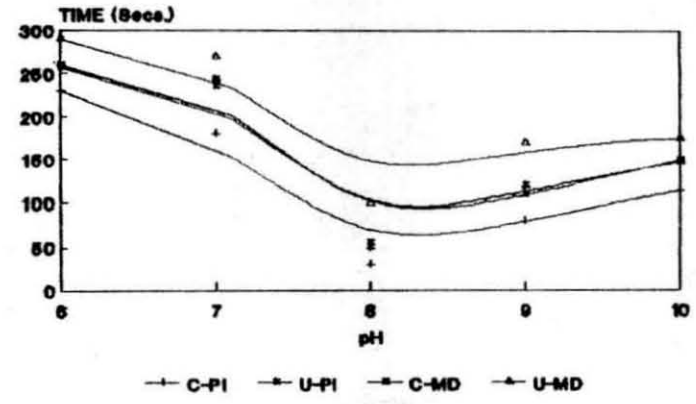
POST-LARVAE



JUVENILE



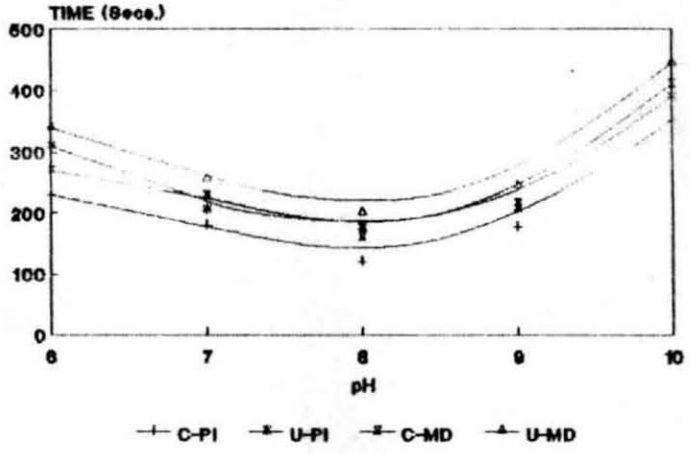
SUB-ADULT



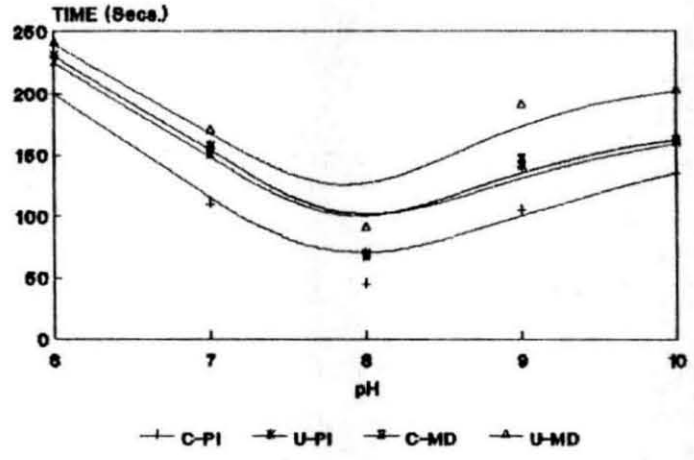
C- Cooked feed; U- Uncooked feed
PI-*Penaeus indicus*; MD- *Metapenaeus dobsoni*

ARRIVAL

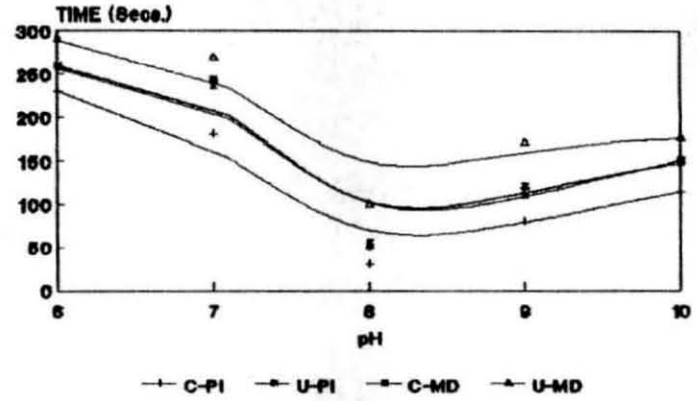
POST-LARVAE



JUVENILE



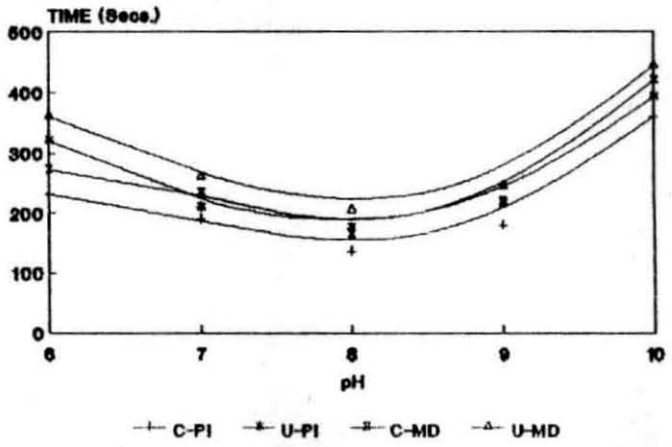
SUB-ADULT



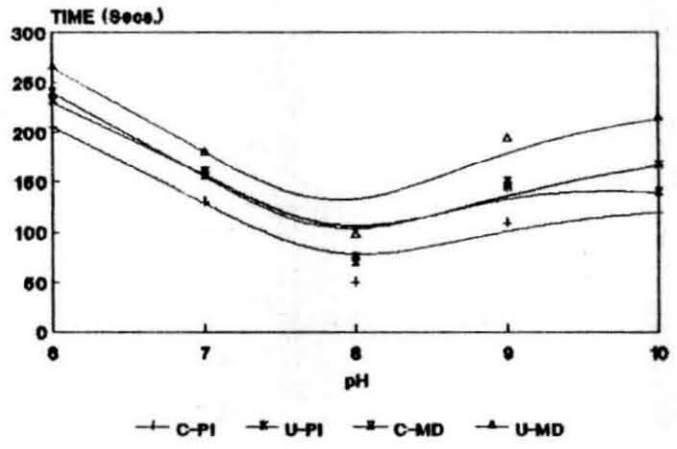
C- Cooked feed; U- Uncooked feed
PI-*Penaeus indicus*; MD- *Metapenaeus dobsoni*

INGESTION

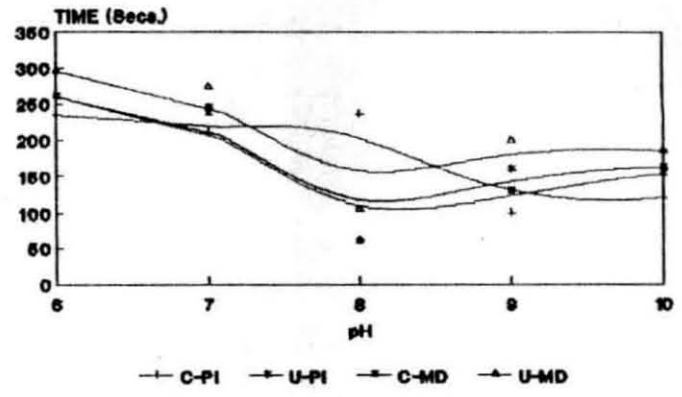
POST-LARVAE



JUVENILE



SUB-ADULT



C- Cooked feed; U- Uncooked feed
PI-*Penaeus indicus*; MD- *Metapenaeus dobsoni*

followed by 9.0 and 7.0. The test animals showed the same degree of response at pH 7.0 and 9.0 with no significant difference in the response time. Among the extreme pH levels tested (6.0 and 10.0) the response of the post-larvae were quick at pH 6.0 than at pH 10.0. But in the case of juveniles and sub-adults the response was rapid at pH 10.0 than at pH 6.0.

The increase in the time lag to elicit feeding response was slow when the pH was raised from 8.0 to 10.0 in the juveniles and sub-adults. When the pH was lowered from 8.0 to the acidic side, upto pH 7.0 the increase in the time lag was gradual, but below 7.0 the increase was very steep except in the case of post-larvae of both species where the time lag increased sharply when the pH was raised from 9.0 to 10.0. This study indicates that acidic pH has pronounced influence on the feeding chemosensory functions of the juveniles and sub-adult shrimps than the alkaline pH and the most ideal pH for food searching and feeding activity being 8.0. But for the post-larvae, alkaline pH was seen to have an adverse effect on chemoreception than the acidic pH. The pH of the rearing medium significally influenced ($P<0.01$) the feeding responses of different stages of both species of shrimps.

The post-larvae, juveniles and sub-adults of both species differed significantly ($P<0.05$) in their feeding responses at different pH levels. Juveniles are chemotactically more responsive as they respond more quickly at all pH levels tested followed by sub-adults. Post - larvae took more time to elicit feeding behaviour than juveniles and sub-adults. At all pH levels studied P.indicus responded more quickly to the feeding stimuli with a shorter time lag than M.dobsoni.

Once the presence of stimulus in the medium was detected by the shrimp the subsequent feeding behaviours occurred in sequence. At pH 8.0 followed by pH 9.0 the time lag between the detection of the stimuli and the expression of the subsequent feeding behaviour was small when compared to other pH levels studied. But this time lag did not differ significantly either between different pH levels or between the different stages of the animals tested. The time lag between two consecutive behaviour was slightly longer in M.dobsoni than P.indicus but did differ at significant levels ($P<0.05$).

When the animals located the source of stimulus the ingestion activity started immediately irrespective of the stages of animals. But the magnitude of these activities depended upon the pH levels. Ingestion behaviour was more intense at pH 8.0 followed by 9.0 and least at 6.0 in the case of juveniles and sub-adults, and for post-larvae it was least at 10.0. The time required to elicit ingestion behaviour in shrimps at different pH levels are given in Fig. 20 (d).

The time lag to elicit ingestion behaviour in 50% of the test animals (ET_{50} values) when an amino acid coated feed was provided is given in table 33. The time lag was minimum at pH 8.0 for post-larvae (89.95 and 100.13 secs), juveniles (82.31 and 95.99 secs) and sub-adults (86.04 and 96.18 secs) of P.indicus and M.dobsoni respectively. In the case of P.indicus post-larvae maximum time lag was observed at pH 10.0 (215.44 secs.) whereas it was maximum at pH 6.0 for M.dobsoni post-larvae (220.74) and the juveniles (177.32 and 183.7 secs.) and sub-adults (189.95 and 204.59 secs.) of P.indicus and M.dobsoni respectively.

3.2.3. **Group Response:** Responses of the shrimp to feeding stimulus at different pH levels studied are presented in Tables 34 and 35 for P.indicus

TABLE 33 : TIME LAG TO LOCATE THE STIMULUS SOURCE BY 50% OF THE TEST ANIMALS AT DIFFERENT pH LEVELS.

	pH				
	6.0	7.0	8.0	9.0	10.0
a. P.indicus					
Post-larvae	205.00	106.0	89.95	97.10	215.4
Juveniles	177.32	98.23	82.31	95.0	115.76
Sub adult	189.93	106.65	86.04	98.12	125.76
b. M.dobsoni					
Post-larvae	220.74	141.96	100.13	132.83	186.52
Juveniles	183.7	115.71	95.99	108.62	151.14
Sub adult	204.59	124.06	96.98	126.81	159.74

TABLE 34 : PERCENTAGE RESPONSE OF PENAEUS INDICUS TO FEEDING STIMULUS DURING 2 MINUTE OBSERVATION AT DIFFERENT pH LEVELS

	pH				
	6.0	7.0	8.0	9.0	10.0
a. Perception					
Post-larvae	30.0	60.03	73.33	63.33	26.67
Juveniles	43.33	83.33	86.67	73.33	50.0
Sub adult	30.0	76.67	83.33	70.0	43.33
b. Ingestion					
Post-larvae	26.67	53.33	56.67	50.0	23.33
Juveniles	33.33	66.67	76.67	66.67	46.67
Sub adult	26.67	60.0	66.67	63.33	26.67

TABLE 35 : PERCENTAGE RESPONSE OF METAPENAEUS DOBSONI TO FEEDING STIMULUS DURING 2 MINUTE OBSERVATION AT DIFFERENT pH LEVELS

	pH				
	6.0	7.0	8.0	9.0	10.0
a. Perception					
Post-larvae	26.67	43.33	66.67	46.67	26.67
Juveniles	33.33	66.67	83.33	63.33	56.67
Sub adult	26.67	63.33	73.33	60.0	43.33
b. Ingestion					
Post-larvae	20.0	36.667	46.67	43.33	16.67
Juveniles	26.67	56.67	63.33	50.0	43.33
Sub adult	20.0	43.33	46.67	46.67	33.33

TABLE 36 : ACTIVITY (IN UNITS) OF TEST ANIMALS AT DIFFERENT pH LEVELS.

pH	P.indicus	M.dobsoni
6.0	2	1
7.0	5	4
8.0	11	8
9.0	8	6
10.0	3	2

and M.dobsoni respectively. During the two minute observation maximum response was observed for juveniles followed by sub-adults and least for the post-larvae. Maximum response was observed at pH 8.0 and least at pH 6.0 in juveniles and sub-adults; whereas least response was observed at pH 10.0 for post-larvae. The responses of the animals varied significantly ($P<0.05$) with varying pH. In the post-larvae of P.indicus 56.7% of the test animals initiated the ingestion activity at pH 8.0, 26.7% at 10.0, and 30.0% at 6.0; in juveniles 86.67% at pH 8.0 and 43.33% at pH 6.0 and in the sub-adults the response was 83.33% at pH 8.0 and 26.67% at pH 6.0 and 10.00 during the two minute period. This response of P.indicus was much higher than that of M.dobsoni under the same condition.

3.2.4. **Quantitative Findings:** The behavioural intensity of the test animals at different pH levels are represented in Fig. 21 and table 36. The intensity and duration of activity was maximum at pH 8.0 followed by pH 9.0 in both species. Maximum activity was recorded at pH 8.0 (11 and 8 units) followed by at pH 9.0 (8 and 6 units) for P.indicus and M.dobsoni respectively. The activity was lowest at pH 6.0 (2 and 1 units). Both P.indicus and M.dobsoni showed a similar pattern of activity at different pH levels, but the magnitude of activity recorded varied considerably, M.dobsoni recording lower values than P.indicus.

3.2.5. **Feeding Response:** The quantity of feed consumed by the shrimps in two hours at different pH levels are presented in table 37. pH influenced the quantity of feed consumed by the test animals significantly ($P<0.01$). The maximum feed consumption was observed at pH 8.0 and minimum at pH 6.0 for juveniles and sub-adults and pH 10.0 in post-larvae for both species. At pH

FIG 21 : Behavioural activity of P.indicus and M.dobsoni towards a stimulus source at different pH levels.

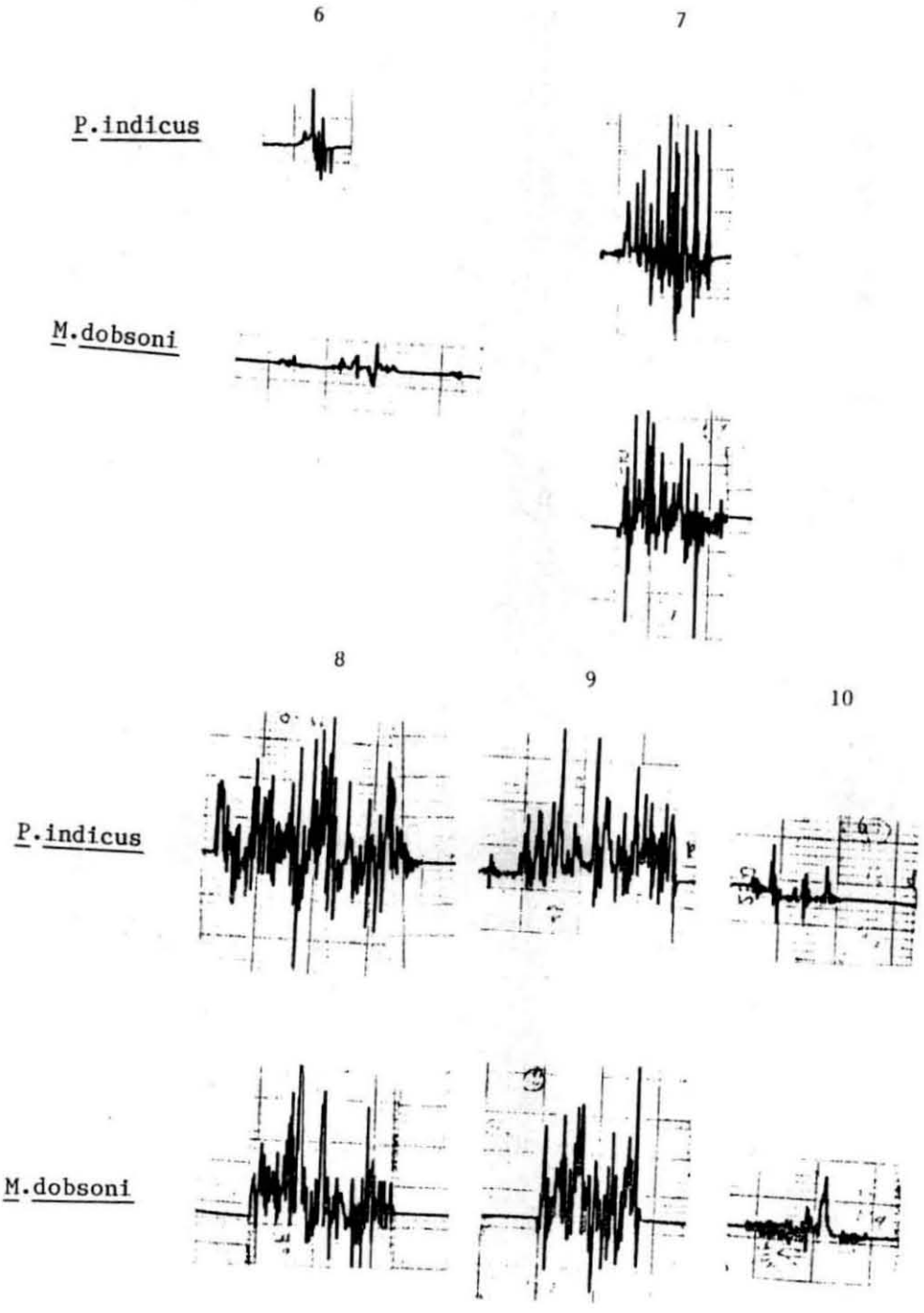


TABLE 37 : FEED CONSUMPTION (GM FEED/100G BODY WEIGHT OF TEST ANIMALS) BY P.INDICUS AND M.DOBSONI AT DIFFERENT pH LEVELS.

	pH				
	6.0	7.0	8.0	9.0	10.0
(a) P.indicus					
(i) Uncoated feed					
Post-larvae	1.275	2.402	2.415	1.859	1.093
Juveniles	0.637	0.893	1.326	0.927	0.797
Sub adult	0.545	0.816	1.203	0.823	0.601
(ii) Glycine coated feed					
Post-larvae	2.075	3.713	3.805	3.193	1.173
Juveniles	0.862	1.257	1.815	1.213	0.895
Sub adult	0.756	1.193	1.235	1.831	0.829
(b) M.dobsoni					
(i) Uncoated feed					
Post-larvae	1.293	1.72	2.034	1.892	1.014
Juveniles	0.506	0.676	1.124	0.619	0.645
Sub adult	0.503	0.721	0.987	0.723	0.527
(ii) Glycine coated feed					
Post-larvae	2.193	3.056	3.521	3.017	1.827
Juveniles	0.861	1.147	1.691	0.820	0.926
Sub adult	0.695	1.095	1.141	0.765	0.751

6.0 and 10.0 the feed consumption was reduced by about 50% than that consumed at pH 8.0.

There was no significant difference in the feed consumption at pH 9.0 and 7.0 and at pH 10.0 and 6.0 ($P<0.05$). A reduction in pH towards the acidic level (pH 6.0) has great influence on feed consumption than when the pH increased towards the alkaline level (pH 10.0) for juveniles and sub-adults. The most ideal pH for better feed consumption in P.indicus and M.dobsoni is 8.0.

This study also showed that the feed coated with a stimulant was more effective in eliciting ingestion than an uncoated feed. The coating of feed with an attractant/stimulant significantly reduced the time required to attract the shrimps to the feed and initiate ingestion in a short time (Fig. 20). Coating of feed significantly increased the amount of feed consumed per unit biomass of the shrimps ($P<0.05$). The feed consumption increased by 57.56% and 48.70% in post-larvae, 36.88% and 56.7% in juveniles and 2.66% and 15.6% in sub-adults of P.indicus and M.dobsoni respectively at pH 8.0 during the 2 hr. observation. Both M.dobsoni and P.indicus differed in the amount of feed consumed, differences were also observed between the post-larvae, juveniles and sub-adults. Between the species studied; P.indicus; and between the stages post-larvae consumed more feed per unit body weight.

4. EFFECT OF STARVATION ON THE FEEDING BEHAVIOUR

4.1 BEHAVIOURAL RESPONSE

Varying degree of starvation significantly influenced the feeding responses and time lag to elicit different feeding behaviour of shrimps ($P<0.05$). With the increase in the degree of starvation, the response

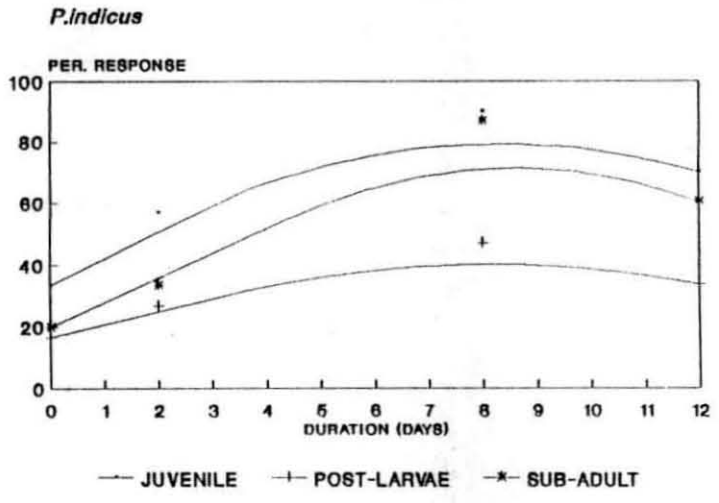
increased initially upto 8 day and there after it decreased (Fig 22). The decline in response was more steep in juveniles than the other groups. The response was high for animals starved upto 8 days. Post-larvae, juveniles and sub-adults showed similar pattern of activity at all levels of starvation. Juveniles showed the highest feeding response at all levels of starvation than post-larvae and sub-adults.

4.2 TIME LAG TO RESPOND

The time lag to elicit perception behaviour initially decreased with the degree of starvation and is minimum for 8 day starved animals (Fig. 23). The time lag there after again increased gradually with the starvation. The time lag to elicit the detection behaviour in a well fed P.indicus and M.dobsoni was 90 and 115 second in post-larvae, 55 and 75 in juveniles and 70 and 85 in sub-adults respectively. But in a 8 day starved animal it was only 50 and 65 (Post-larvae), 10 & 35 (juveniles) and 20 and 50 (sub-adults) seconds respectively for P.indicus and M.dobsoni. The time lag gradually increased thereafter and showed a maximum of 115 and 135, 80 and 105 and 85 and 135 seconds for post-larvae, juveniles, and sub-adults of P.indicus and M.dobsoni respectively.

The time lag to locate the stimulus source and to initiate ingestion activity depics the same pattern as that of perception. Time lag was minimum for 8 day starved animals and maximum for well fed animals (Fig. 24). The time lag between the initiation of perception behaviour and ingestion activity also varied with degree of starvation. It was minimum for 8 day starved animals and maximum for well fed and highly starved animals.

FIG 22 : Percentage response of post-larvae, juveniles and sub-adults of P.indicus and M.dobsoni starved for different durations towards stimulus source.



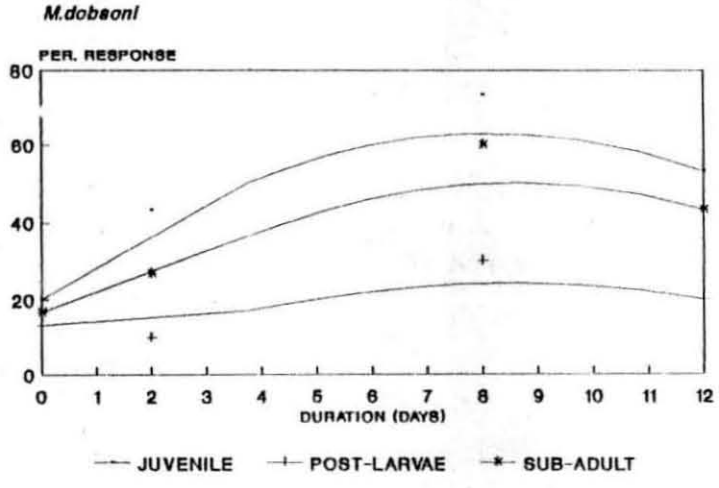


FIG 23 : Latency to elicit perception behaviour by post-larvae, juveniles and sub-adults of P.indicus and M.dobsoni starved for varying durations.

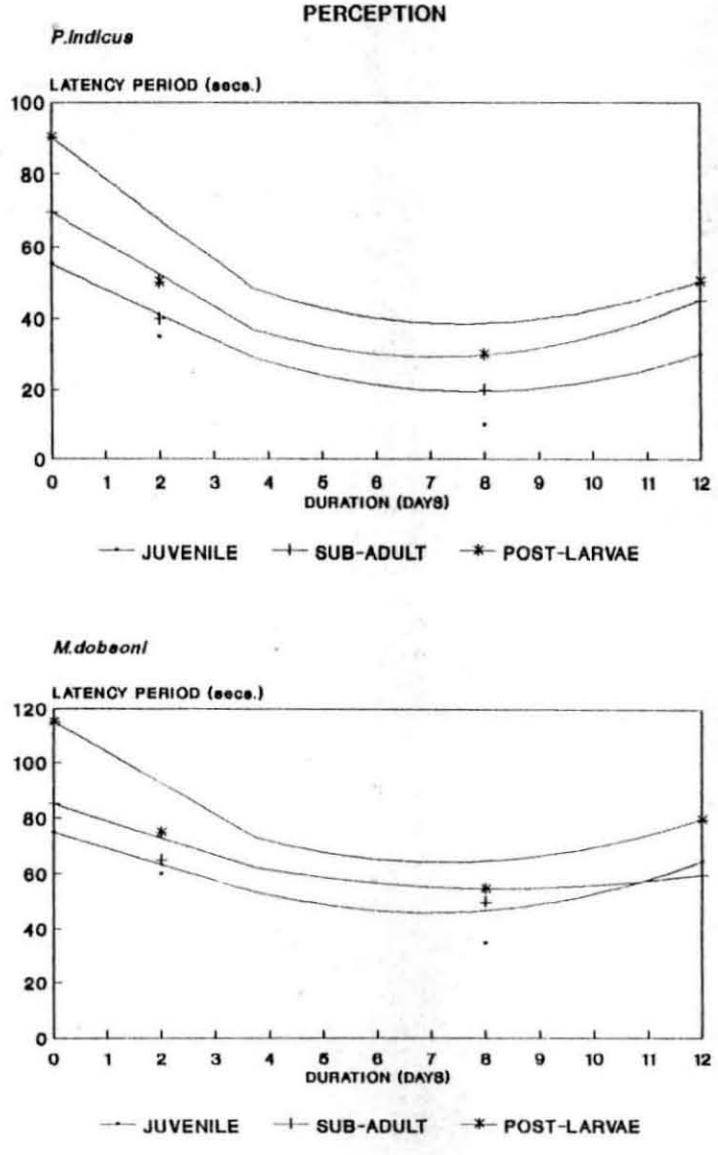
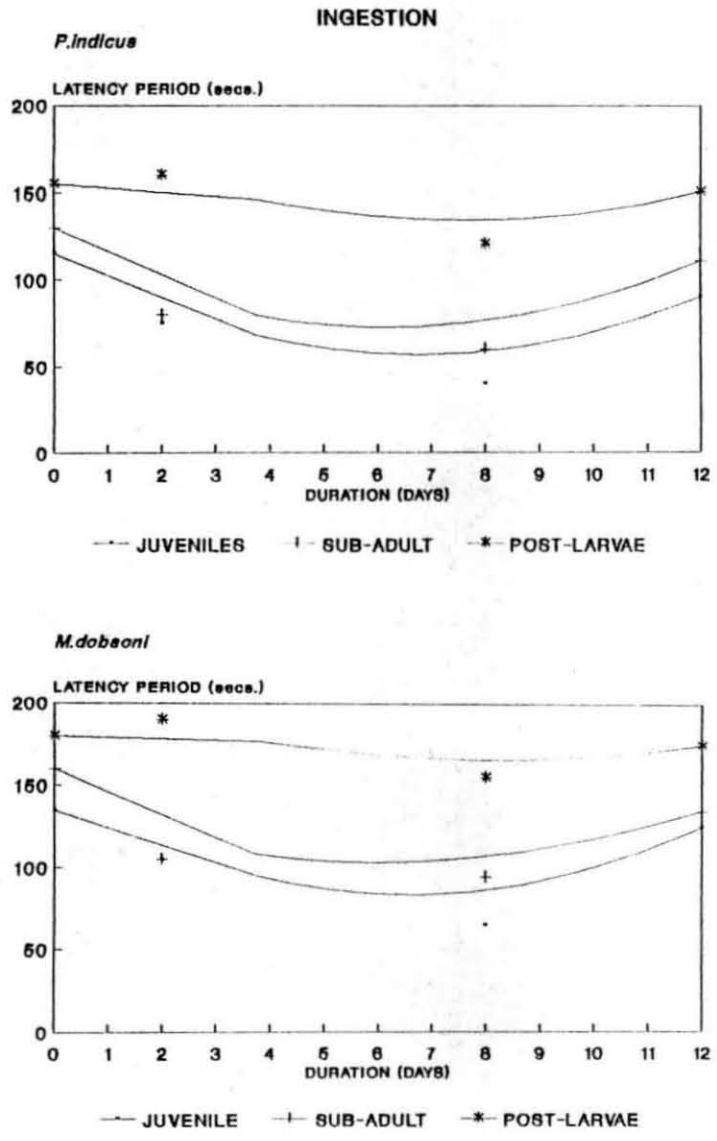


FIG 24 : Latency to initiate ingestion activity by post-larvae, juveniles and sub-adults of P.indicus and M.dobsoni starved for varying durations.



4.3 FEEDING RESPONSE

The feed intake of the test animals varied considerably with degree of starvation (Table 38), ($P<0.05$). The feed intake initially increased and later on declined gradually with starvation rate. Maximum feed intake was observed for 6 day starved animal and minimum for well fed followed by 12 day starved animals.

5. FEEDING CHEMORECEPTOR SITES

5.1. LOCATION OF THE CHEMORECEPTOR SITES

The results of the study are summarized in tables 39 and 40. It indicated that the major sites involved in feeding chemoreception are inner and outer ramous of antennule, mouth parts, including maxilliped-III and the chelae of the first three pairs of walking legs. The chemosensory cilia present on these sites are involved in the detection of the chemical stimuli.

5.2. EVALUATION OF THE CHEMORECEPTOR SITES

Results of this study are summarized in Tables 41 and 42, which represent the behavioural responses of the shrimps with all appendages and body parts except the test appendage being exposed and with the test appendage alone being exposed. Fig 25 (a-e) represents the time lag to elicit various feeding behaviours when the test appendage was exposed or not exposed. The time lag increased significantly compared to the control when the the antennule, periopod chelae, periopods, and mouth parts were blocked ($P<0.01$). The maximum increase in the time lag was observed when the antennule alone was blocked followed by periopods, chelae of the first three pairs of walking legs and the mouth parts blocked. No change in the latency to elicit feeding behaviour was observed when other sites were blocked.

TABLE 38 : FEED INTAKE (GM FEED/100 GM BODY WT.) BY THE TEST ANIMALS AT VARIOUS LEVELS OF STARVATION.

	Duration of starvation (days)					
	0	2	4	6	8	12
P.indicus						
Post-larvae	0.122	3.170	3.840	4.140	3.870	3.130
Juveniles	0.100	1.870	2.070	2.410	2.120	1.680
Sub-adults	0.130	0.870	1.110	1.130	1.090	0.864
M.dobsoni						
Post-larvae	0.094	2.940	3.180	3.630	2.410	2.390
Juveniles	0.091	1.690	1.740	1.980	1.860	1.640
Sub-adults	0.220	0.974	1.220	1.230	1.190	0.864

TABLE 39 : RESPONSES OF THE APPENDAGES AND GENERAL BODY SURFACE TO THE TEST STIMULI.

(a) P.indicus

Test organ/ Body Parts	Response of the Animal										Total Positive Response
	1	2	3	4	5	6	7	8	9	10	
Rostrum	-	-	-	-	-	-	-	-	-	-	0
Eye	-	-	-	-	-	-	-	-	-	-	0
Antennule (Inner Ramous)	+	+	+	+	+	+	+	+	+	+	10
Antennule (Outer Ramous)	+	+	+	+	+	+	+	+	+	+	10
Antennae	-	-	-	-	-	-	-	-	-	-	0
Mouth parts (Excluding Maxilliped 3)	+	+	+	+	+	+	+	+	+	+	10
Maxilliped 3	-	-	-	-	+	-	-	+	-	+	3*
Gills	-	-	-	-	-	-	-	-	-	-	0
Periopods											
Chelae 1	+	+	+	+	+	+	+	+	+	+	10
Chelae 2	+	+	+	+	+	+	+	+	+	+	10
Chelae 3	+	+	+	+	+	+	+	+	+	+	10
Propodus + Dactylus (4-5)	-	-	-	-	-	-	-	-	-	-	0
Merus + carpus (1)	+	+	+	-	-	+	-	-	+	+	6
Merus + carpus (2-5)	-	-	-	-	-	-	-	-	-	-	0
Basis (1-5)	-	-	-	-	-	-	-	-	-	-	0
Pleopods (1-5)	-	-	-	-	-	-	-	-	-	-	0
Pleopod basis (1-5)	-	-	-	-	-	-	-	-	-	-	0
Uropod	-	-	-	-	-	-	-	-	-	-	0
Telson	-	-	-	-	-	-	-	-	-	-	0
Abdominal surface	-	-	-	-	-	-	-	-	-	-	0
Carapace	-	-	-	-	-	-	-	-	-	-	0

* Only weak response was observed.

TABLE 40 : RESPONSES OF THE APPENDAGES AND GENERAL BODY SURFACE TO THE TEST STIMULI.

M.dobsoni

Test Appendage/ Body Parts	Response of the Animal										Total Positive Response
	1	2	3	4	5	6	7	8	9	10	
Rostrum	-	-	-	-	-	-	-	-	-	-	0
Eye	-	-	-	-	-	-	-	-	-	-	0
Antennule (Inner Ramous)	+	+	+	+	+	+	+	+	+	+	10
Antennule (Outer Ramous)	+	+	+	+	+	+	+	+	+	+	10
Antennae	-	-	-	-	-	-	-	-	-	-	0
Mouth parts (Excluding Maxilliped 3)	+	+	+	+	+	+	+	+	+	+	10
Maxilliped 3		+			+	+		+			4*
Gills	-	-	-	-	-	-	-	-	-	-	0
Periopods											
Chelae 1	+	+	+	+	+	+	+	+	+	+	10
Chelae 2	+	+	+	+	+	+	+	+	+	+	10
Chelae 3	+	+	+	+	+	+	+	+	+	+	10
Propodus + Dactylus (4-5)	-	-	-	-	-	-	-	-	-	-	0
Merus + carpus (1)	+	-	-	+	+	+	-	+	+	+	7
Merus + carpus (2-5)	-	-	-	-	-	-	-	-	-	-	0
Basis (1-5)	-	-	-	-	-	-	-	-	-	-	0
Pleopods (1-5)	-	-	-	-	-	-	-	-	-	-	0
Pleopod basis (1-5)	-	-	-	-	-	-	-	-	-	-	0
Uropod	-	-	-	-	-	-	-	-	-	-	0
Telson	-	-	-	-	-	-	-	-	-	-	0
Abdominal surface	-	-	-	-	-	-	-	-	-	-	0
Carapace	-	-	-	-	-	-	-	-	-	-	0

* Only weak response was observed.

TABLE 41 : FUNCTION OF CHEMORECEPTOR SITES ON BEHAVIOURAL PARAMETERS RELATED TO FEEDING RESPONSE IN PENAEUS INDICUS

	BEHAVIOUR*						
	I	II	III	IV	Va	Vb	Vc
Animals without :							
1. Vision (5)	+	+	+	+	+	+	-
2. Antennae (5)	+	+	+	+	+	+	-
3. Antennule (5)	P	P	P	P	P	P	-
4. Pereopods (1st) 3 pairs (5)	+	-	+	+	-	-	-
5. Pleopods (5)	+	+	+	+	+	+	-
6. Chelae coated (5)	P	P	P	+	-	-	+
7. Mouth parts (5)	+	+	+	+	-	-	+
8. Control (5)	+	+	+	+	+	+	-
Animals with :							
1. Vision only (5)	-	-	-	-	-	-	+
2. Antennae only (5)	-	-	-	-	-	-	+
3. Antennule only (5)	+	+	+	+	-	-	+
4. Pereopods only (5)	P	P	P	P	+	-	+
5. Pleopods only (5)	-	-	-	-	-	-	+
6. Chelae exposed only (5)	P	P	P	P	+	-	+
7. Mouth parts only (5)	P	P	P	P	-	-	+
8. Only gills exposed (5)	-	-	-	-	-	-	+

Number of animals used has been shown in parenthesis
P - Poor Response

* Behaviours have been explained in the text.

TABLE 42 : FUNCTION OF CHEMORECEPTOR SITES ON BEHAVIOURAL PARAMETERS RELATED TO FEEDING RESPONSE IN METAPENAEUS DOBSONI

	BEHAVIOUR*						
	I	II	III	IV	Va	Vb	Vc
Animals without :							
1. Vision (5)	+	+	+	+	+	+	-
2. Antennae (5)	+	+	+	+	+	+	-
3. Antennule (5)	P	P	P	P	P	P	-
4. Pereopods (1st 3 pairs) (5)	+	-	+	+	-	-	-
5. Chelae coated (5)	P	P	P	+	-	-	+
6. Mouth parts (5)	+	+	+	+	-	-	+
7. Control (5)	+	+	+	+	+	+	-
Animals with :							
1. Vision only (5)	-	-	-	-	-	-	+
2. Antennae only (5)	-	-	-	-	-	-	+
3. Antennule only (5)	+	+	+	+	-	-	+
4. Pereopods only (5)	P	P	P	P	+	-	+
5. Pleopods only (5)	-	-	-	-	-	-	+
6. Chelae exposed (5)	P	P	P	P	+	-	+
7. Mouth parts only (5)	P	P	P	P	-	-	+
8. Only gills exposed (5)	-	-	-	-	-	-	+

Number of animals used have been shown in paranthesis.
P - Poor Response

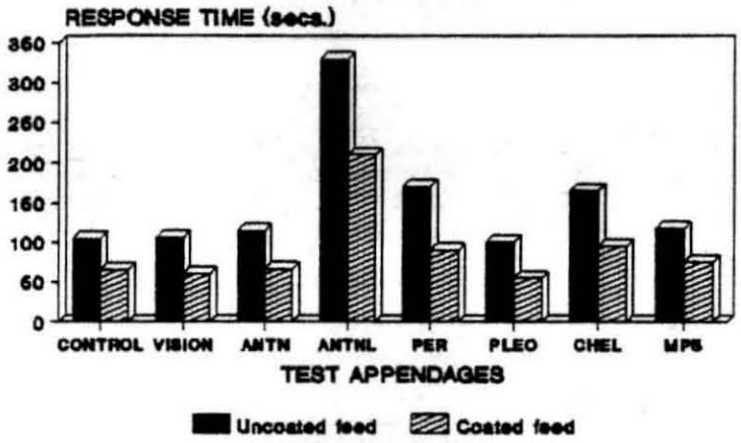
* Behaviours have been explained in the text.

FIG 25 : Time lag to elicit various feeding behaviours by P.indicus and M.dobsoni when the test appendages were exposed or blocked.

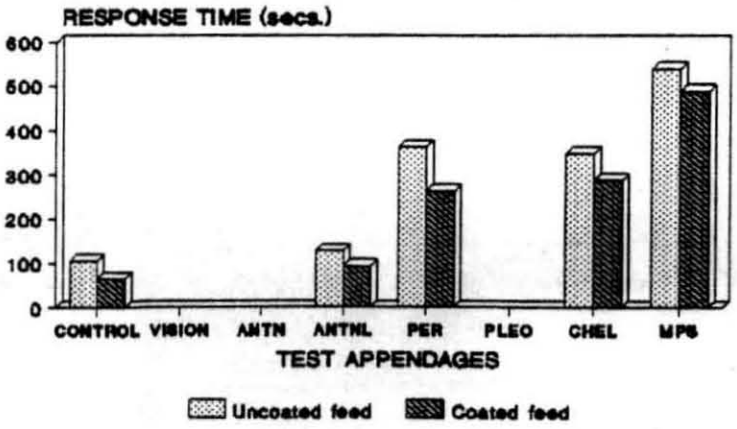
(A) PERCEPTION

Test appendage not exposed

P.indicus



Only test appendage exposed

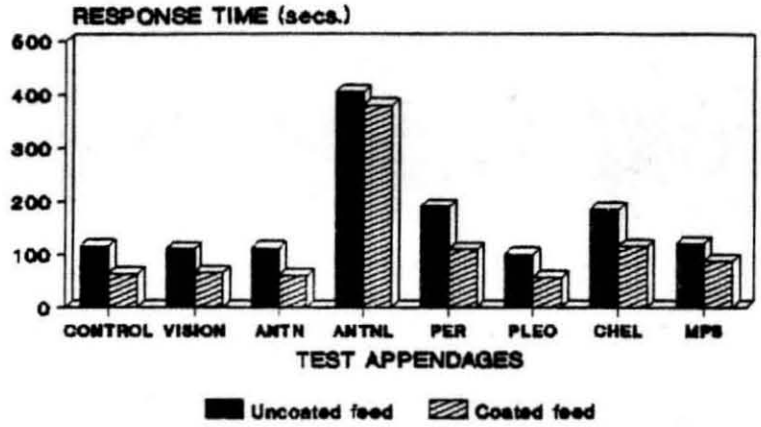


Blank space indicates no feeding activity

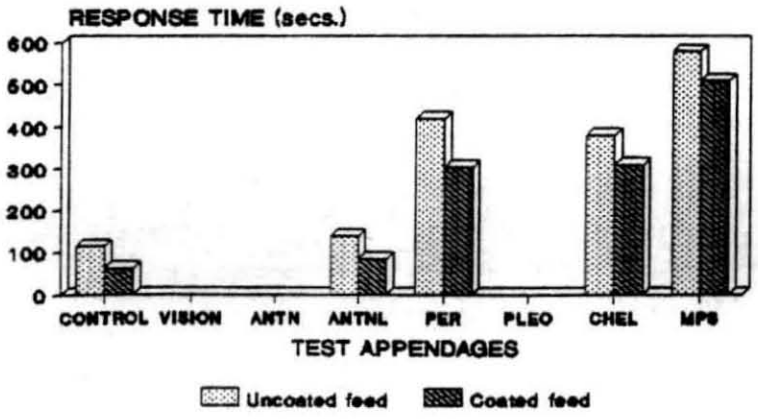
(A) PERCEPTION

Test appendage not exposed

M.dobsoni



Only test appendage exposed

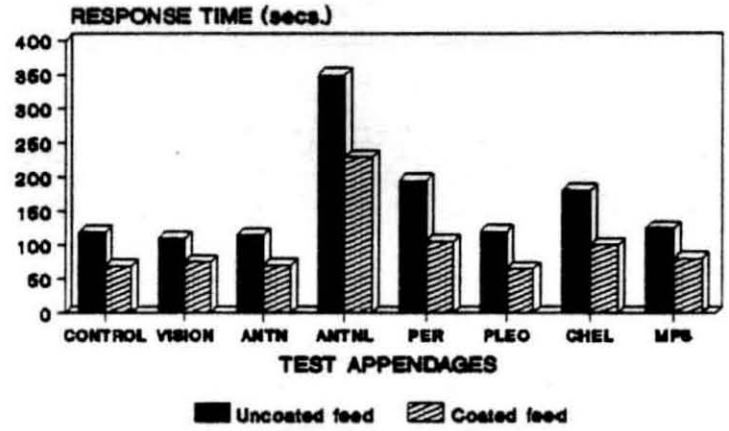


Blank space indicates no feeding activity

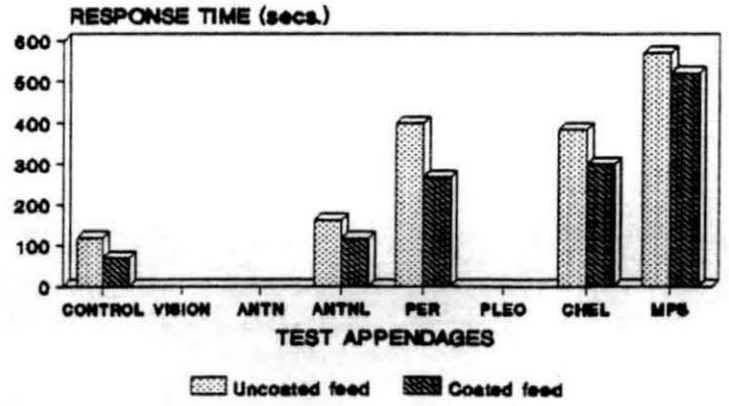
(B) ORIENTATION

Test appendage not exposed

P.Indicus



Only test appendage exposed

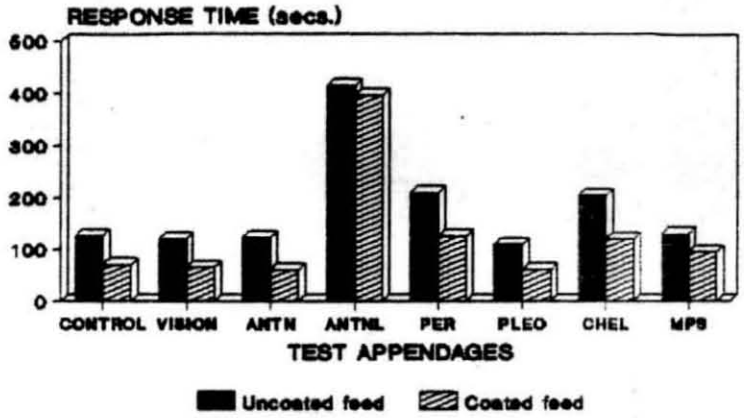


Blank space indicates no feeding activity

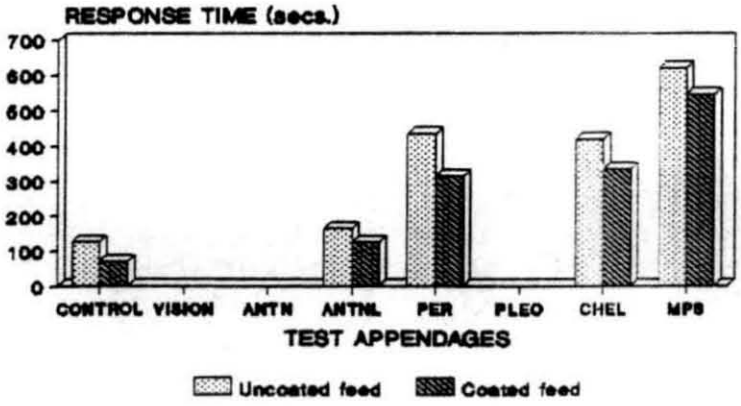
(B) ORIENTATION

Test appendage not exposed

M.dobsoni



Only test appendage exposed

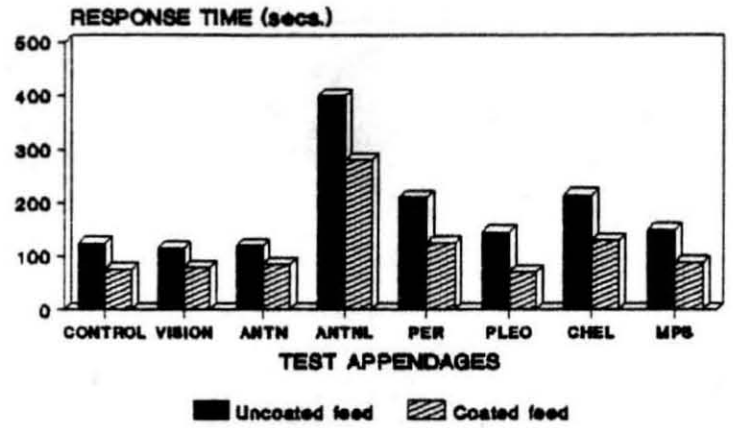


Blank space indicates no feeding activity

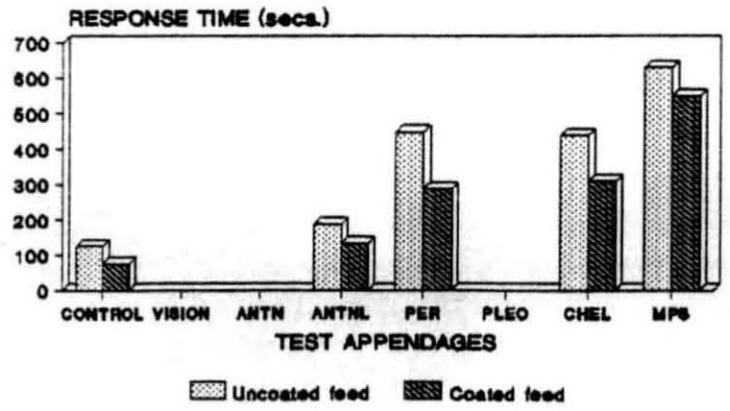
(C) DISPLACEMENT

Test appendage not exposed

P.indicus



Only test appendage exposed

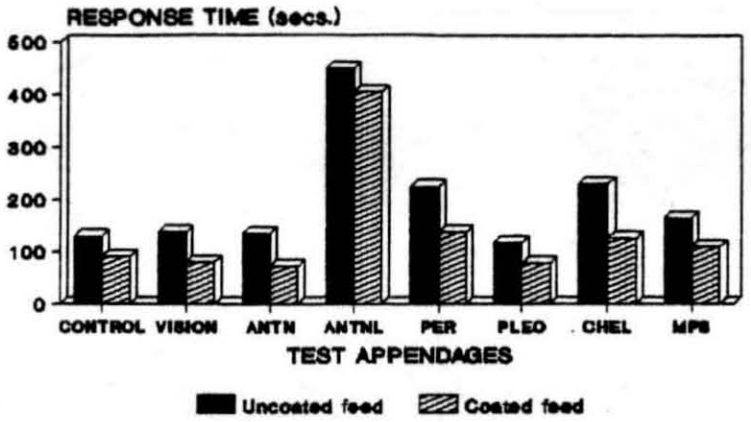


Blank space indicates no feeding activity

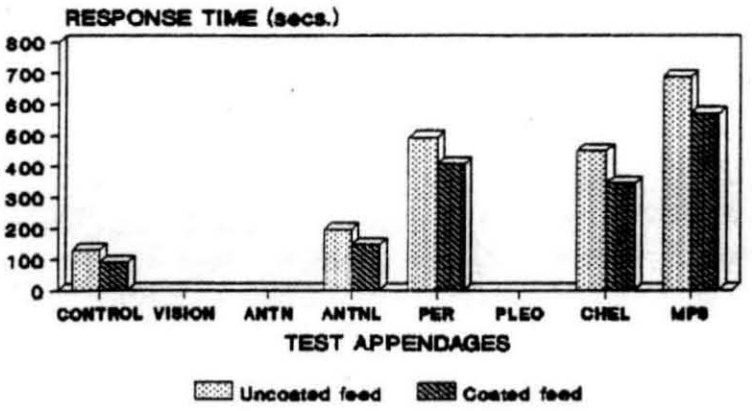
(C) DISPLACEMENT

Test appendage not exposed

M.dobsoni



Only test appendage exposed

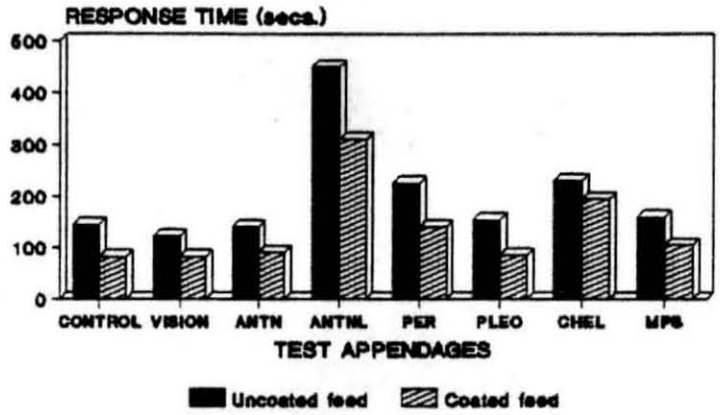


Blank space indicates no feeding activity

(D) ARRIVAL

Test appendage not exposed

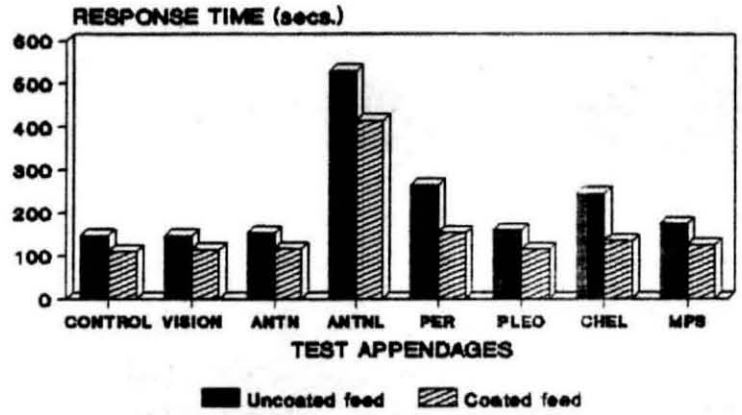
P.indicus



(D) ARRIVAL

Test appendage not exposed

M.dobsoni



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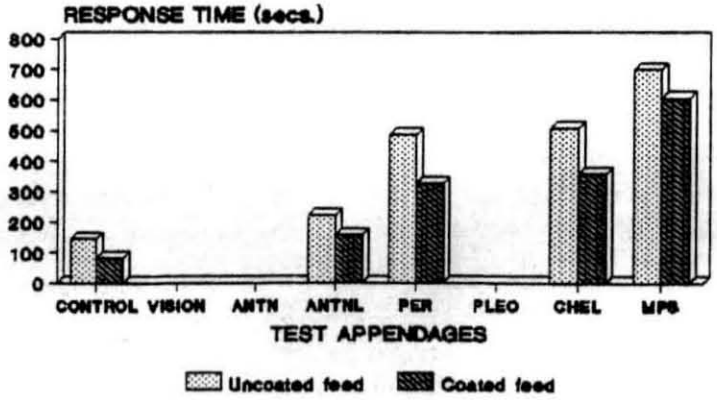

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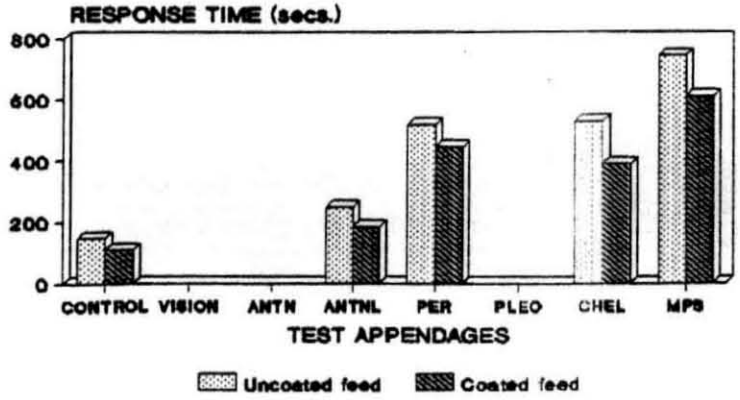


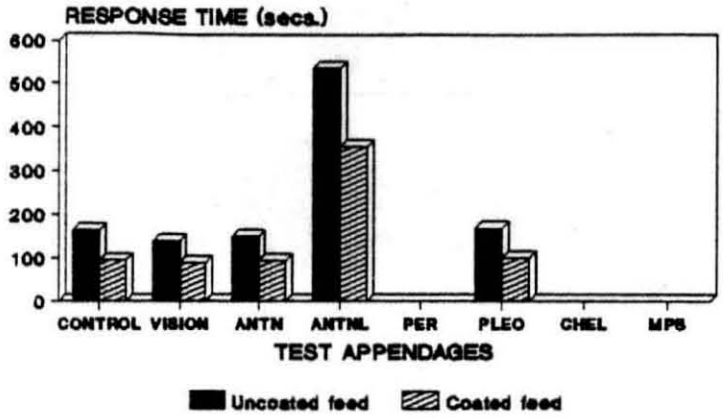
Blank space indicates no feeding activity

Blank space indicates no feeding activity

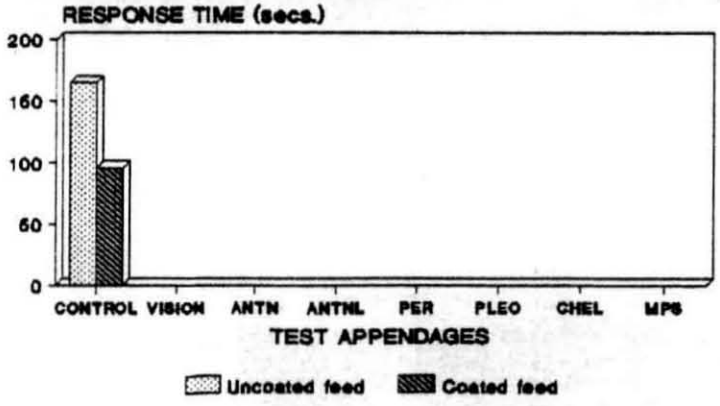
(E) INGESTION

Test appendage not exposed

P.indicus



Only test appendage exposed

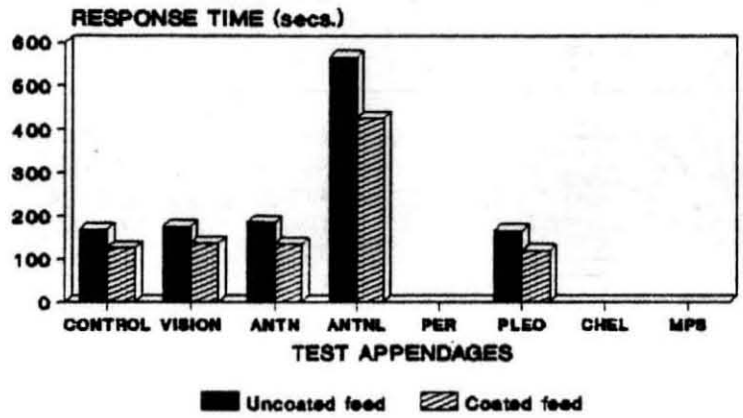


Blank space indicates no feeding activity

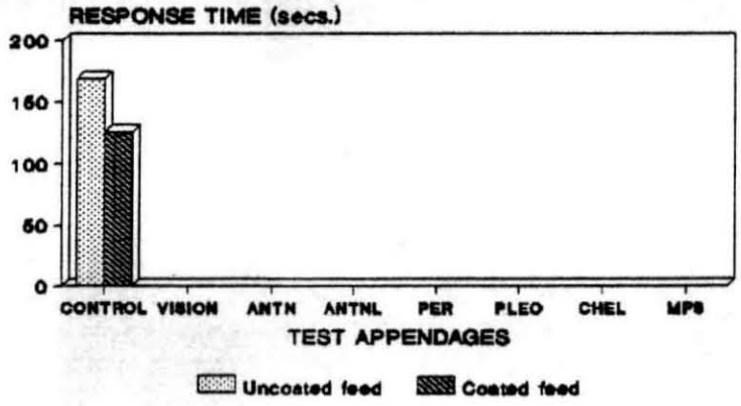
(E) INGESTION

Test appendage not exposed

M.dobsoni



Only test appendage exposed



Blank space indicates no feeding activity

When the test appendage alone was exposed to the feeding stimuli, response was observed when the antennule, periopod chelae, first three pairs of periopods Maxilliped-III and mouth parts were exposed. Time lag to elicit feeding response was minimum when the antennules alone were exposed, followed by first three pairs of periopods exposed, periopod chelae exposed, mouth parts and Maxilliped-III alone exposed. No feeding response was observed when other appendages alone were exposed.

The time required to elicit the feeding response in 50% (Et_{50}) of the test animals were given in Table 43. When the test appendage alone blocked the large ET_{50} value (136.85 & 141.281 secs) was recorded for antennule, followed by periopods (120.51 & 115.77 secs) chelae of periopods (111.33 & 112.36 secs) and mouth parts (85.52 & 78.57 secs) blocked animals and in the case of all other appendages and sites it was same as that for control (73.5 & 76.55 secs) respectively for P.indicus and M.dobsoni. When the test appendage alone was exposed, antennule gave the small ET_{50} value followed by periopods, chelae of the periopods and mouth parts. For other sites studied, no response was obtained during the 10 minute observation.

5.3. STRUCTURE AND FUNCTION OF CHEMORECEPTOR SITES

This study indicated that antennules, chelae of the first three pairs of walking legs, and mouth parts including maxillipeds are the major chemoreceptor sites involved in the detection of feed stimuli.

5.3.1. **Vision:** The shrimps that are devoid of vision alone did not depict any significant increase in the latency period nor any change in the feeding behaviour, compared to the control ($P<0.05$). When the food was placed in front of the test animal with vision alone exposed did not show any feeding

TABLE 43: TIME LAG TO LOCATE THE STIMULUS SOURCE BY 50% OF THE TEST ANIMALS.

	CONTROL	VISION	ANTENNA	ANTENNULE	PEREOPODS	PEREOPOD CHELAE	MOUTH PARTS	PLEOPODS	MAXILLIPED-III
A. TEST APPENDAGE NOT EXPOSED									
P.indicus	73.497	73.99	76.54	136.85	120.51	111.33	86.31	73.03	74.17
M.dobsoni	76.55	77.24	77.09	141.28	115.765	112.36	78.57	76.93	78.00
B. TEST APPENDAGE EXPOSED									
P.indicus	NR*	NR*	NR*	121.78	172.47	180.8	372.96	NR*	448.6
M.dobsoni	NR*	NR*	NR*	115.437	221.99	245.19	456.32	NR*	496.7

* N.R. : No response during 10 minute observation.

activity during the 10 minute observation. But they approached the feed pellets, at a later time, without exhibiting any feeding behaviour. This observation shows that the shrimps detect their food mainly with the help of olfactory sites and not by visual cues (Fig.25).

5.3.2. **Antennae:** When the antennae alone were blocked the feeding behaviour of the animals remained the same without any significant change in the ET_{50} value compared to control ($P<0.05$). When the antennae were alone exposed no feeding response occurred indicating no significant role of antennae in chemoreception.

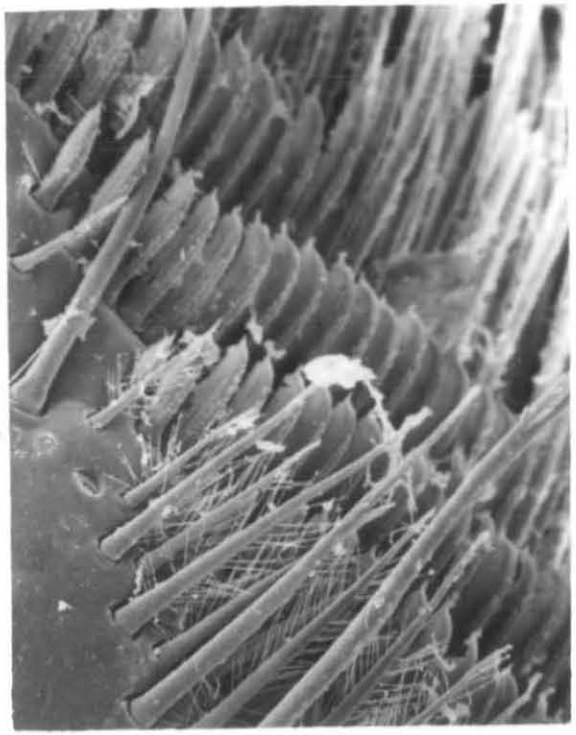
5.3.3. **Antennule:** Antennule consists of three basal segments and a pair of multijointed flagilla or ramii. Both the inner and outer ramous are involved in chemoreception (Table 41 and 42). Blocking of the antennule significantly increased the time lag to detect the stimuli (210 and 380 secs for P.indicus and M.dobsoni respectively) and to locate its source (310 and 415 secs) compared to that of control(Fig 25), ($P<0.05$). But when the antennules alone were exposed, the time lag became smaller of the order of 95 and 85 seconds for detection and 160 and 185 for location of the stimuli source respectively for P.indicus and M.dobsoni. Antennules play a significant role in the perception of the chemical stimulus and functions as a distance chemoreceptor as indicated by sharp increase in the ET_{50} value when blocked and the small ET_{50} value when exposed alone (Table 43).

The light and scanning electron microscopic observations revealed different types of setae distributed along the rami (plate 1 and 2) and along the basal segments (plates 3,4 and 5). Two types of setae were observed along the ramous arising from the junction of the segments. One type is short and sturdy and narrow towards the distal end with a flat tip (plate 1). The other

PLATE 1 : Scanning Electron Micrograph (SEM) showing the origin and structure of single seta on ramous of antennule (x 320).

PLATE 2 : Scanning Electron Micrograph showing the tubular porous setae with sensory pore (note the tip) enabling chemoreception (x 2500).

PLATE 3 : SEM showing setae distributed over the surface of basal segment of the antennule (x 320).



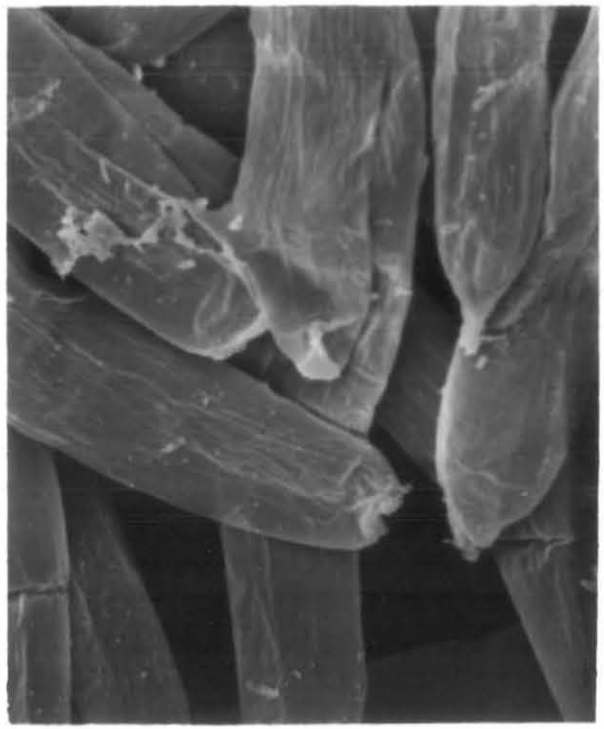
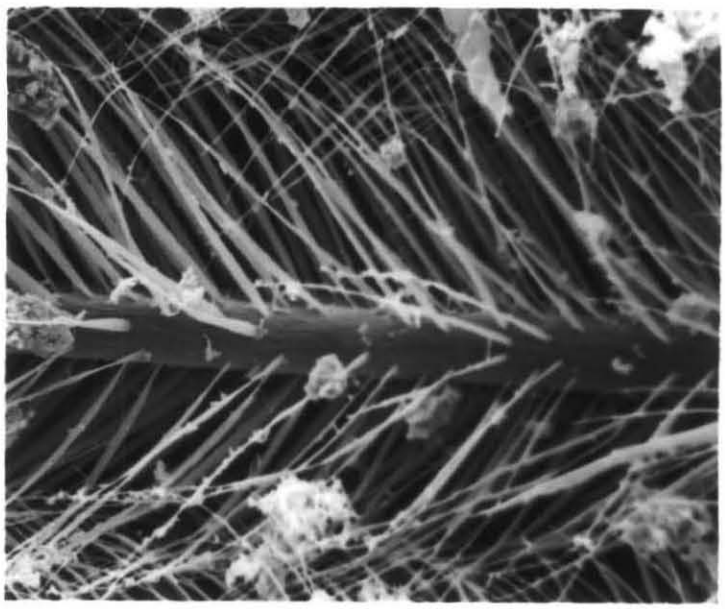
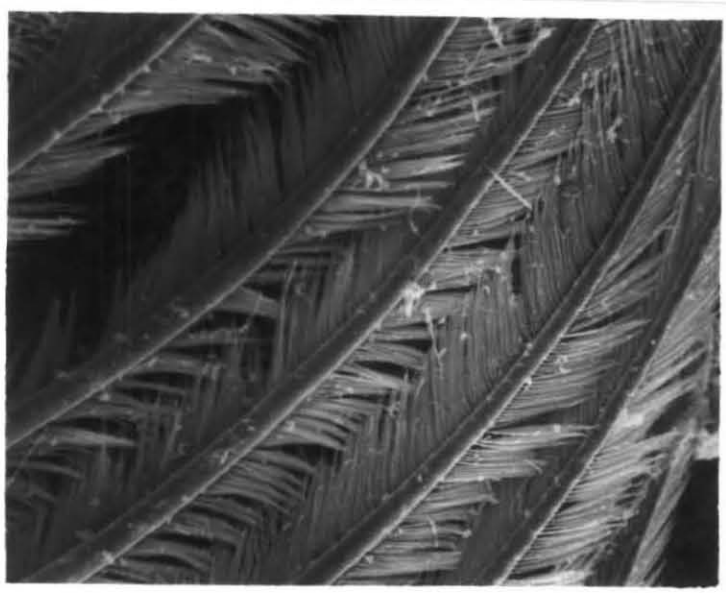


PLATE 4 & 5 : Scanning Electron Micrograph showing different Setae types distributed along the margin of the basal segments of the antennule - arrangement of the setae is such that maximum area is exposed in aiding easier perception. (x 1200) .





type of setae is long, membranous, having uniform thickness through out (plate 2). Both the setae types are tubular in structure with a "sensory pore" starting from the tip. It is probable that the latter setae are involved in distance chemoreception and is mainly responsible for perception and orientation. The basal segments also bear simple tubular setae along with branched feather like setae (plate 3). The secondary cilia of the feather like setae may increase the contact area and efficiency of chemoreception (plates 4 and 5), but at present its role is not well known.

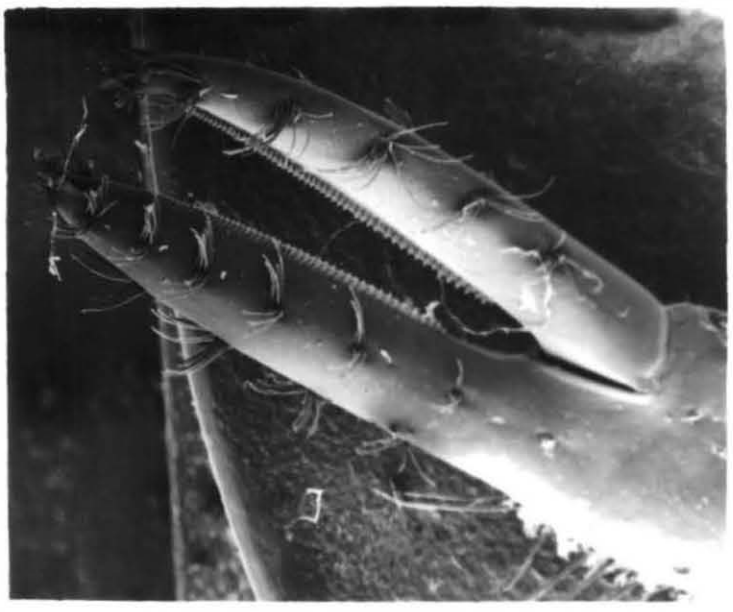
5.3.4. **Pereopods And Chelae** : Blocking of the first three pairs of pereopods or their chelae alone increased the time lag to respond (Fig 25). The ET_{50} value when the whole pereopod was blocked, was slightly higher than that of the chelae alone when blocked (Table 43). When exposed alone ET_{50} was small for pereopods than when the chelae alone were exposed; but both are very large compared to the intact control. When the pereopod or the chelae alone were blocked, the food picking activity totally stopped, and no feeding occurred. But when they were exposed alone, food picking alone occurs, but no mastication and ingestion occurs. These indicate that the pereopods and the chelae act both as a contact and distance chemoreceptor and play a major role in identification, location, food picking and ingestion.

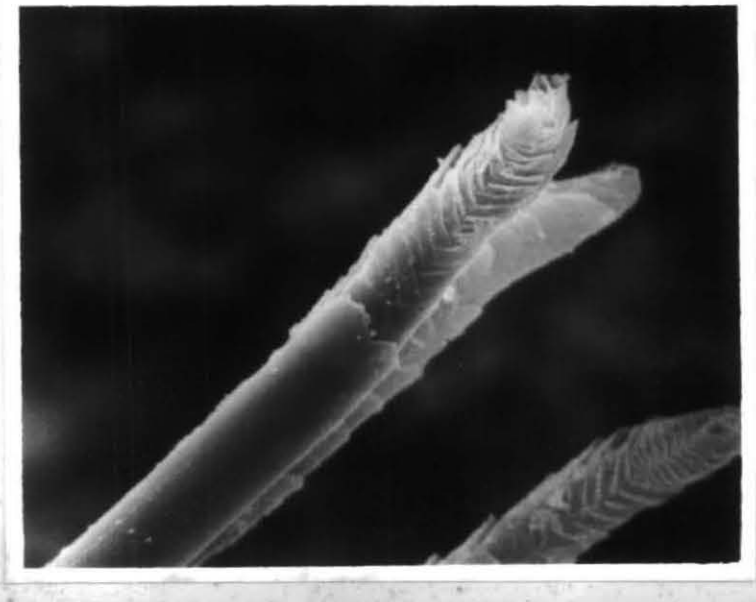
The first three pairs of pereopods are similar in thickness. Shrimps systematically search the substratum and remove small particles of food transferring the same to the mouth using the pereopod chelae. Electron microscopic observations revealed three types of complex cuticular structures on the propus and dactyl sensory setae, a system of ridge and pegs on the inner apposable surface of the chelae and apposable pads of denticles at the tip of the chelae (plate 6).

PLATE 6 : Scanning Electron Micrograph of inner surface of chelae of Pereopod 1, showing relative positions of simple setae (s), pegs (P) and denticulate pads (x 40).

PLATE 7 : Scanning Electron Micrograph of elaborately sculptured simple seta of the distal end of dactylus-pereopod (x 640).

PLATE 8 : Scanning Electron Micrograph of elaborately sculptured tip of a simple seta of the proximal end of the dactylus of pereopod with the pore (x 320).





Simple Setae: Tufts of simple setae are more or less regularly spaced over the surface of the chelae. Distally these groups of setae lie close together, but the distribution of the group and the number of setae in each group become sparser proximally. The simple setae of dactyl is tubular, but differ slightly in their structure. The tip of the setae occurring at the distal region of chelae is elaborately sculptured into a series of disc-like plates, surrounding and presumably protecting a sub-terminal pore, within which may be seen the termination of an internal structure which is probably sensory (plate 7). In the case of setae of the proximal region of the chelae, immediately below the sensory pore there are several pairs (14-15 pairs) of vertically placed plates, which meet in the mid line (plate 8). The position and structure of these setae suggested that they are involved in contact chemoreception and is responsible for discriminating edible from non-edible materials.

Pegs and Ridges: The jaws of each chelae are lined with crenellated ridges bearing regularly spaced pegs which extend from the distal tip almost to the propus-dactylus articulation (plate 9). Pegs are seen to arise from the depression at the base of the ridges and have no pores, either at the tip or anywhere on the surface. It is probable that these are mechanorceptive devices with a particle size discriminating function.

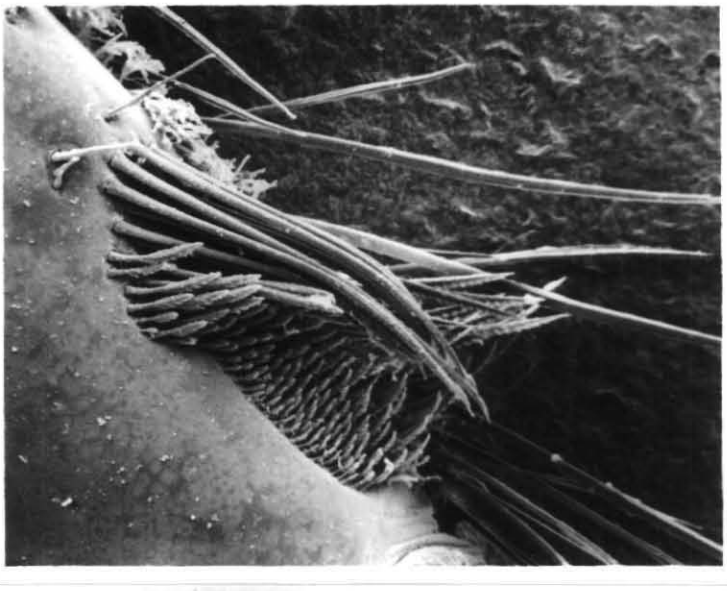
Denticulate Pads: It comprises of a large tuft of proximally angled denticles; appears to be a device for firmly gripping rather than testing small objects (plate 10). The denticles are solid in nature and are formed as an extension of the cuticle.

At the articulation of the propus and carpus of the first pereopod there is a system of four types of setae (plate 11). Of this the three groups of

PLATE 9 : Scanning Electron Micrograph of pegs (P) inserted into the lipped depressions (L) of the chelate pereopod (x 640).

PLATE 10 : Scanning Electron Micrograph of tip of chela of pereopod I showing the origin of setae (x 640) (note the pore).

PLATE 11 : SEM of junction of the propus and carpus of pereopod I showing the 4 distinct groups of cleaning setae (x 80).



setae seen on the inner surface of the chelae are involved in cleaning other chelate pereopods. (plates 12,13 and 14). One group of setae which lies close to the proximal end of the propus on the inner surface has a similar structure with their upper halves expanded into an upward pointing lateral extension, but they decrease in height both proximally and towards the upper surface (plate 12). Two other types of setae form another group on the inner surface of the distal end of the carpus. The first type in long (plate 13) and the second type short (plate 14), with upward pointing lateral extensions.

Another group of branched setae arising from the lower surface of the distal end of the carpus near the inner side just behind the cleaning setae (plate 15). They have a tubular structure with a sensory pore at the distal end of each branch. It is probable that these are distance chemoreceptors, responsible for perception and orientation which observed in the absence of antennule.

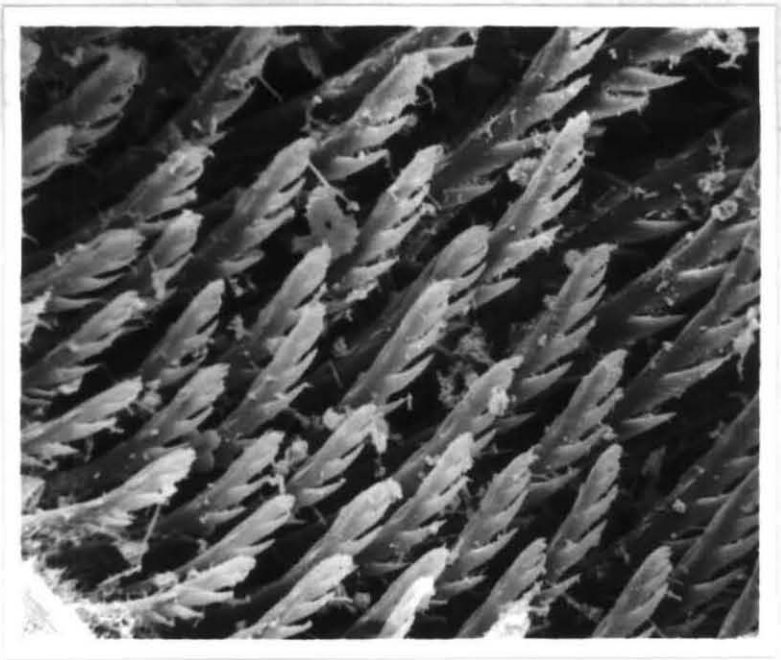
5.3.5. **Mouth Parts:** The time lag to elicit feeding behaviour increased (97 and 110 secs respectively for P.indicus and M.dobsoni) when the mouth parts were blocked, than in the intact control (65 and 63 secs) (Fig 25). Though the pereopods, pushed the food towards the mouth no mastication occurred. When the mouth parts alone were exposed, the time lag further increased (490 and 510 secs) and though the animal located the stimulus source, no food picking activity occurred. The chelate legs and the mouth parts when exposed together, the time lag further decreased, and ingestion activities such as food picking and mastication occurred. These observations indicated that mouth parts have both distance and contact chemoreceptor property.

When the maxilliped-III alone was exposed, the time lag was more than all the mouth parts together exposed, and when it was blocked alone, the time lag

PLATE 12 : Scanning Electron Micrograph showing seta type I (cleaning setae) distributed at the junction of the propus and carpus of the pereopod (x 640).

PLATE 13 : Scanning Electron Micrograph showing seta type II (cleaning setae) distributed at the junction of the propus and carpus of the pereopod (x 640).

PLATE 14 : Scanning Electron Micrograph showing seta type III (cleaning setae) distributed at the junction of the propus and carpus of the pereopod (x 640).



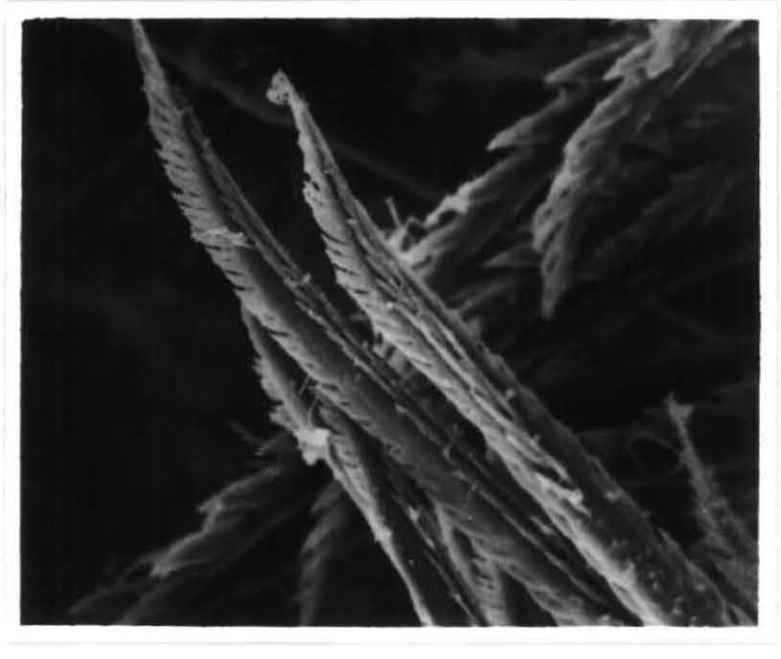
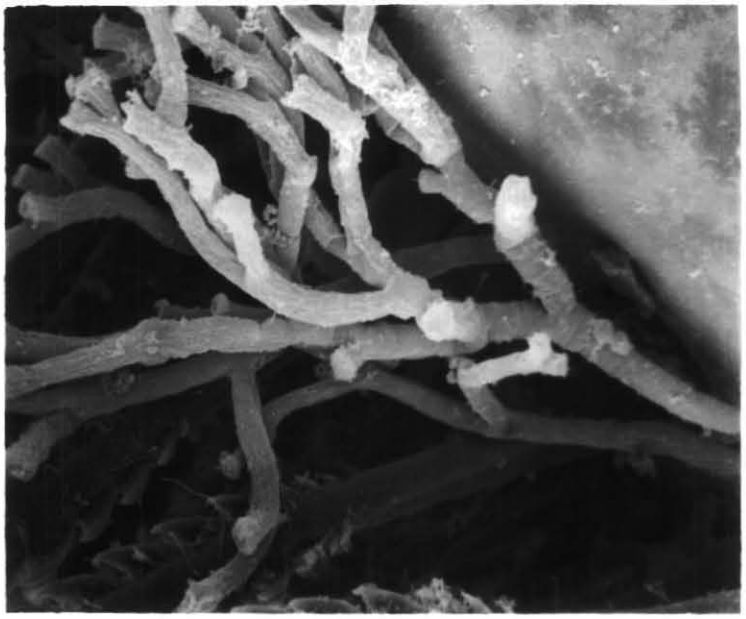
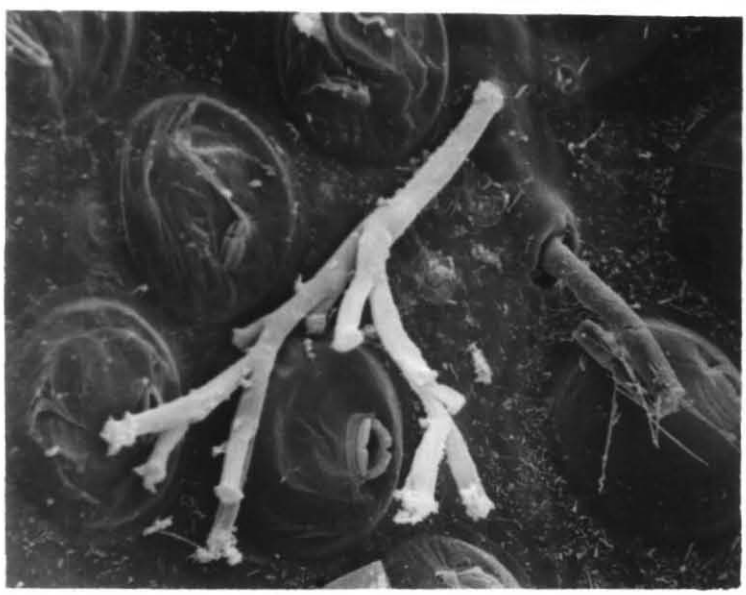


PLATE 15 : Scanning Electron Micrograph showing sensory seta at the lower surface of the carpus-propus junction.

PLATE 16 : Cleaning/sensory setae distributed along the margin of the mandibular palp of the mandible (x 80).

PLATE 17 : Oral pores showing origin of the sensory seta of the mandibular palp (x 640).





to respond slightly increased than the control, but was smaller than when all the mouth parts together were blocked (Table 43). In the absence of maxilliped-III, food picking and ingestion occurred, but at comparatively slower rate. These observations indicated that maxilliped-III is a poor chemoreceptor. But it helps the chelate leg in tranferring food materials to the mouth and cleaning other appendages like first pereopod, mouth parts and antennule.

Mandible: The Mandibular palp bear two types of setae; closely packed with dense secondary cilia along the margin of the palp (Plate 16) and evenly spaced multibranched tubular setae arising from the oral pores on the dorsal surface of the palp (plate 17 and 18). The distal end of the branches of tubular setae has a terminal pore; indicating that they are involved in chemoreception. The former groups of setae may acts as an efficient filter for microscopic food materials and increase the effective surface area available for chemoreception.

Maxilla I and II: They are small, flat and curved to fit closely below the mandibles. The protopod of the maxillule bear densely arranged short and stumpy setae (plate 19). The structure and close association of it with the mandible suggested that they are contact chemoreceptors. Exopodite bear long feather like setae along the margin, which may have the role of filtration of microscopic food particles (plate 20).

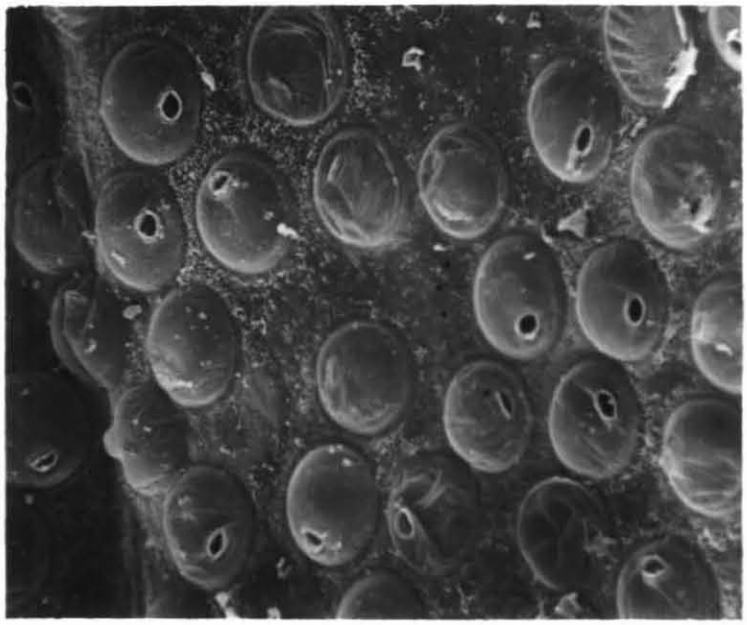
Maxilliped: Maxillipeds are thoracic appendages and become more leg like posteriorly .

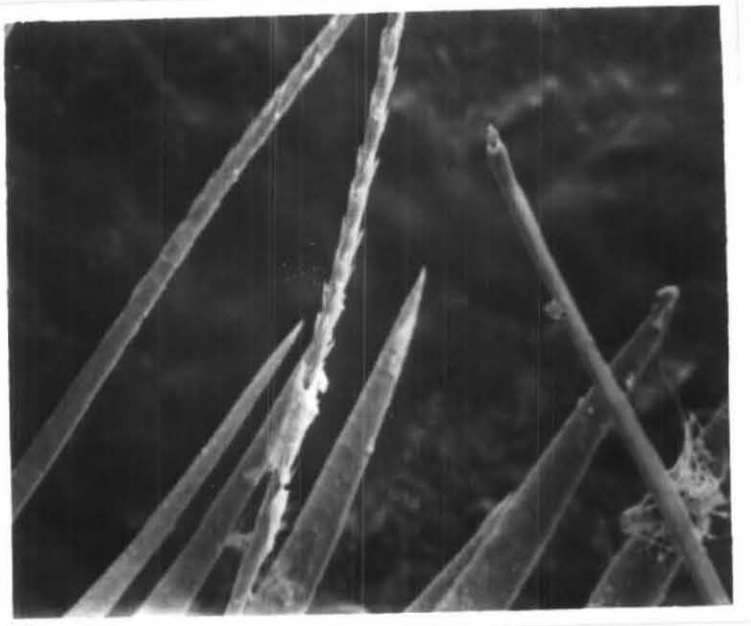
Maxilliped I: Propus bear dense fringing spine like setae to towards its distal end (plate 21). These are tubular setae with a small pore at the distal end. Setae with spine like projections but without pores were alo seen with

PLATE 18 : Oral pores distributed on the upper surface of the mandibular palp (x 320).

PLATE 19 : Scanning Electron Micrograph showing distribution of short, stumpy setae on the maxillule (x 320)

PLATE 20 : Scanning Electron Micrograph showing origin of setae on scaphognathite of maxillule (x 640).





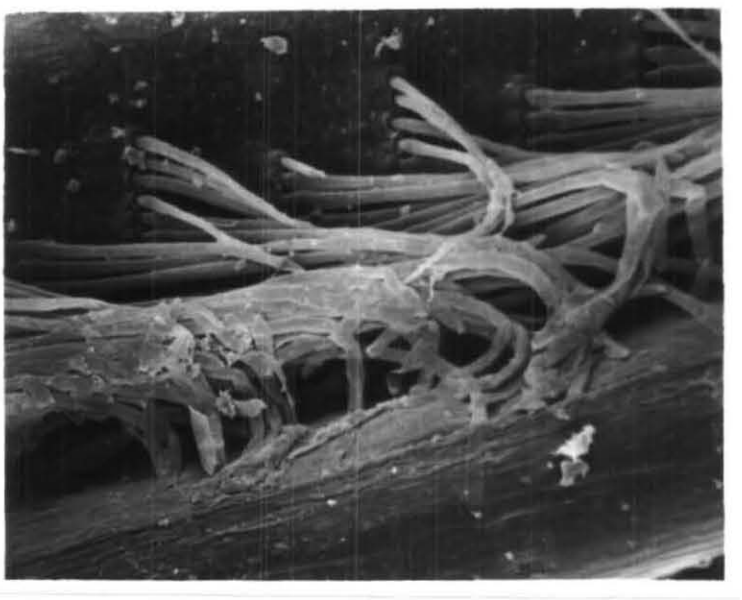


PLATE 21 : Scanning Electron Micrograph of maxilliped I showing three setae types (x 320)-note seta type that has the sensory proe

PLATE 22 : Scanning Electron Micrograph showing setae types distributed on maxilliped I (x 160), branching arrangement of setae provide efficient feeding surface.

PLATE 23 : Scanning Electron Micrograph - origin and structure of the sensory setae of maxilliped II (x 320)

these setae. Two more setae types were observed towards the distal end of the propus and in carpus (plate 22).

Maxilliped II: The distal end of propus bears a longitutional groove. They bear groups of long slender setae on both side of the groove (plate 23). The structure of this setae suggests that they are typical distance chemoreceptors involved in perception.

Maxilliped III: Propus and dactyl bear different types of cleaning setae in groups, such as comb like setae (plate 24) along with setae having structure similar to that of the cleaning setae of the pereopods (plate 25). The scattered short stumpy spine like setae, observed along this region may seem to have some chemosensory role (plate 26). Exopodite is a long structure formed of several segments with highly settuled setae on each side which are supposed to involved in filter feeding (plate 27).

Epipodites: The epipodites of all thoracic appendages were shown to have similar structure and bear only one type of setae which may be involved in respiration (plate 28).

Pleopods: Pleopods when exposed alone showed no feeding response and when blocked, no significant change was noticed in the feeding activity ($P<0.05$).

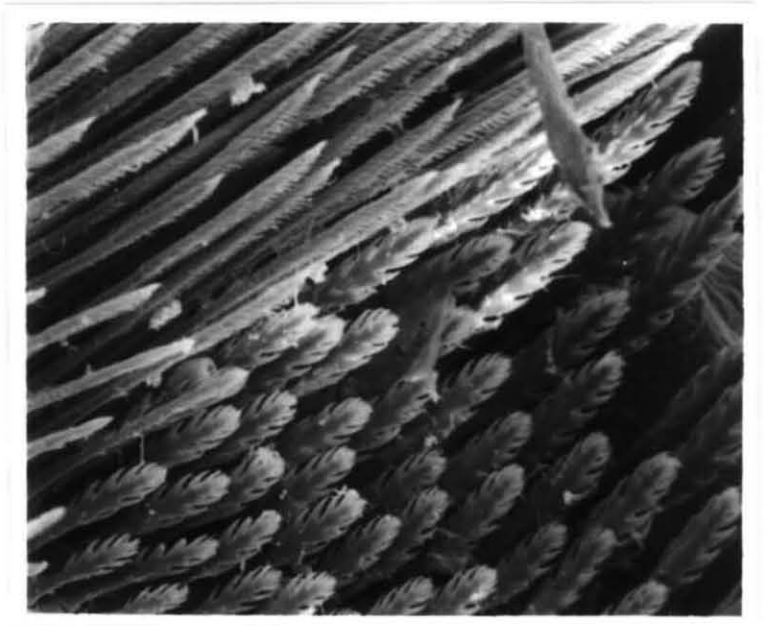
5.3.7. **Other Body Parts:** Blocking and exposure of other body parts such as rostrum, telson and uropods produced the same response as pleopods, indicating their insignificant feeding chemosensory function.

The presence of a very weak feeding response observed at a later stage when all the non-chemosensory sites were exposed or when all the above test appendages were blocked together, indicate the presence of some other chemosensory sites like ciliary lining of gills.

PLATE 24 : Scanning Electron Micrograph shows setae types of maxilliped III (x 320)

PLATE 25 : Scanning Electron Micrograph shows setae types of maxilliped III (x 640)

PLATE 26 : Scanning Electron Micrograph shows the different setae types distributed on maxilliped III (x 320)



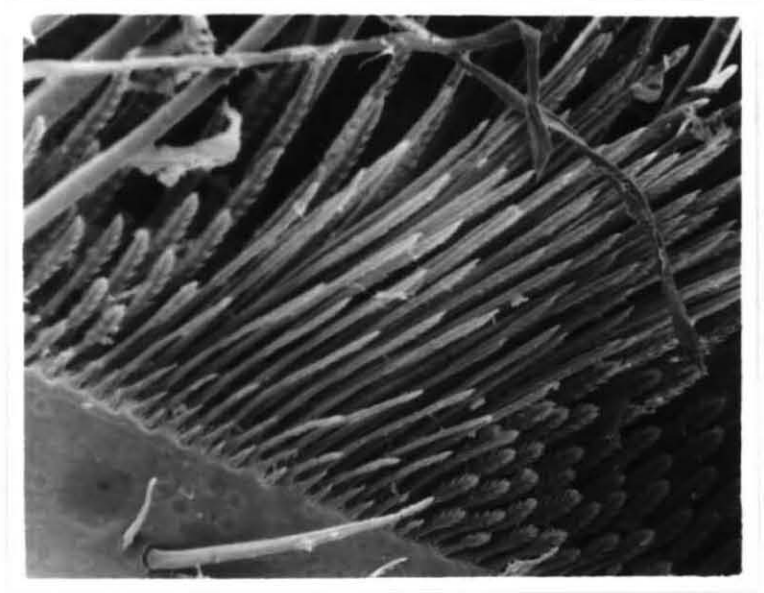


PLATE 27 : Scanning Electron Micrograph showing the setae type of exopodite of maxilliped III (x 40)

PLATE 28 : Setae of epipodite of maxilliped III (x 250)

6. INGESTIVE PROPERTY OF NATURAL AND CHEMICAL STIMULI

Tissue extracts were assayed for their feeding stimulus activity in shrimps, the results of which are presented in Tables 44 and 45. Unflavoured agar gel elicits little feeding activity by itself, while agar flavoured with tissue extracts had higher palatability as indicated by the higher consumption rates in both the species. Agar consumption varied significantly for different tissue types ($P<0.01$) indicating variation in their stimulatory efficacy. P.indicus and M.dobsoni varied in their affinity to different test samples. In all trials, P.indicus preferred agar gel flavoured with squid extract followed by crab, clam, oyster and shrimp extracts. M.dobsoni preferred agar gel flavoured with squid, shrimp, clam, crab and oyster extracts in the order of preference. P.indicus showed least preference to gel flavoured with seaweed extract and M.dobsoni to the gel flavoured with earthworm extract. In both the species, agar gel consumption increased significantly with the test sample concentration ($P<0.05$).

Incorporation of squid ink in the gel decreased its palatability. The agar consumption decreased with the increase in the concentration of squid ink in the gel and at higher concentration, the gel was totally avoided.

Various extract fractions were assayed to determine the tissue component that is responsible for eliciting ingestion activity in shrimps. Details of agar consumption flavoured with different extract fractions are represented in Figs. 26 and 27 and their relative potencies in table 46. The gel flavoured with the whole extract showed the same pattern of preference as stated above. But the preference towards extract fractions varied from that of whole extracts in both species. Maximum agar consumption occurred when flavoured with whole extracts. Removal of the lipid fraction lead to a nominal reduction

TABLE 44 : AGAR GEL CONSUMPTION (g/100 g. BODY WEIGHT OF SHRIMP) BY *PENAEUS INDICUS* IN A TWO CHOICE FEEDING PREFERENCE TEST

TEST STIMULUS	STIMULUS CONCENTRATION (% W/V)					
	0	0.5	1.0	1.5	2.0	2.5
	REFERENCES					
Shark	0.321	0.232	0.336	0.396	0.529	0.788
Mussel	0.035	0.233	0.438	0.521	0.645	0.817
Cuttle Fish	0.032	0.268	0.373	0.483	0.612	0.825
Squilla	0.031	0.126	0.337	0.473	0.613	0.753
Fish	0.035	0.218	0.402	0.495	0.537	0.794
Crab	0.034	0.340	0.426	0.605	0.739	0.988
Shrimp	0.036	0.266	0.440	0.505	0.696	0.842
Black Clam	0.032	0.298	0.422	0.639	0.718	0.948
Squid	0.034	0.336	0.439	0.607	0.779	0.998
Earthworm	0.032	0.169	0.219	0.393	0.448	0.680
Mysid	0.033	0.149	0.313	0.424	0.565	0.603
White Clam	0.032	0.128	0.352	0.467	0.545	0.784
Seaweed	0.034	0.102	0.263	0.310	0.465	0.564
Oyster	0.036	0.274	0.418	0.593	0.712	0.883
Squid (with ink sac)	0.030	0.180	0.116	0.076	0.037	0.023

TABLE 45 : AGAR GEL CONSUMPTION (g/100 g. BODY WEIGHT OF SHRIMP) BY *METAPENAEUS DOBSONI* IN A TWO CHOICE FEEDING PREFERENCE TEST

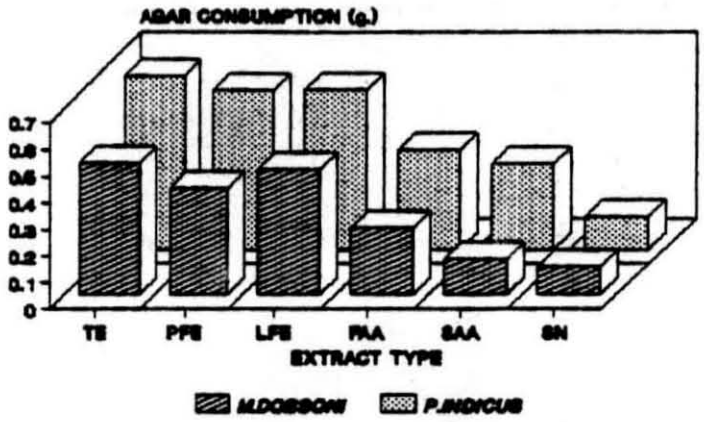
TEST STIMULUS	STIMULUS CONCENTRATION (% W/V)					
	0	0.5	1.0	1.5	2.0	2.5
	REFERENCES					
Shark	0.061	0.295	0.351	0.395	0.404	0.523
Mussel	0.059	0.269	0.418	0.533	0.667	0.734
Cuttle Fish	0.062	0.259	0.414	0.503	0.600	0.698
Squilla	0.062	0.234	0.364	0.428	0.553	0.628
Fish	0.058	0.227	0.367	0.431	0.567	0.634
Black Clam	0.059	0.390	0.442	0.596	0.744	0.882
Oyster	0.064	0.314	0.437	0.534	0.683	0.786
Shrimp	0.061	0.382	0.492	0.603	0.783	0.886
Squid	0.063	0.483	0.534	0.687	0.723	0.899
Earthworm	0.062	0.218	0.310	0.346	0.436	0.505
Mysid	0.065	0.254	0.321	0.353	0.439	0.517
White Clam	0.065	0.296	0.316	0.413	0.529	0.593
Seaweed	0.065	0.263	0.308	0.352	0.416	0.511
Squid (with ink sac)	0.061	0.114	0.103	0.083	0.023	0.000

TABLE 46 : INGESTIVE POTENCY OF TISSUE EXTRACT FRACTIONS AND SYNTHETIC FRACTIONS ON PENAEUS INDICUS AND METAPENAEUS DOBSONI

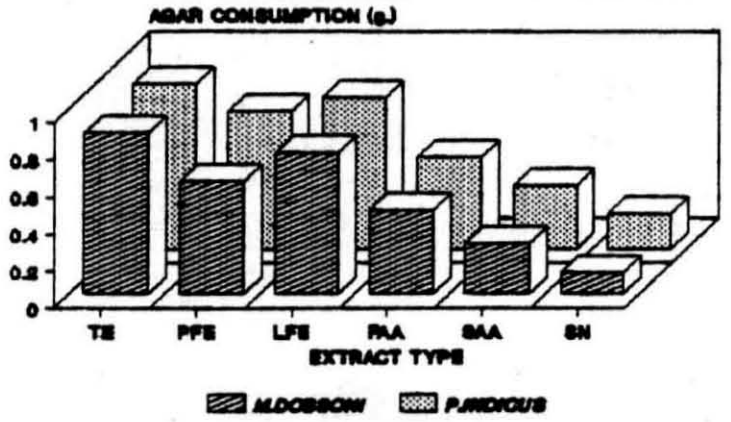
Extract source	TE	LFE	PFE	FAA	SAA	SN
a. P.indicus						
Fish	1.0	0.950	0.807	0.506	0.273	0.215
Clam	1.0	0.877	0.705	0.529	0.316	0.127
Shrimp	1.0	0.927	0.848	0.455	0.319	0.156
Crab	1.0	0.987	0.923	0.572	0.390	0.174
Oyster	1.0	0.931	0.844	0.489	0.312	0.151
Squid	1.0	0.942	0.858	0.522	0.335	0.158
Mussel	1.0	0.958	0.734	0.508	0.320	0.166
Squilla	1.0	0.896	0.798	0.527	-	-
b. M.dobsoni						
Fish	1.0	0.923	0.920	0.580	0.495	0.192
Clam	1.0	0.917	0.834	0.559	0.386	0.217
Shrimp	1.0	0.981	0.903	0.520	0.421	0.261
Crab	1.0	0.973	0.874	0.566	0.434	0.247
Oyster	1.0	0.928	0.843	0.475	0.385	0.182
Squid	1.0	0.894	0.826	0.501	0.357	0.217
Mussel	1.0	0.964	0.863	0.526	0.349	0.168
Squilla	1.0	0.913	0.790	0.86	-	-

FIG 26 : Agar consumption by P.indicus and M.dobsoni when flavoured with different extract fractions from selected tissue sources.

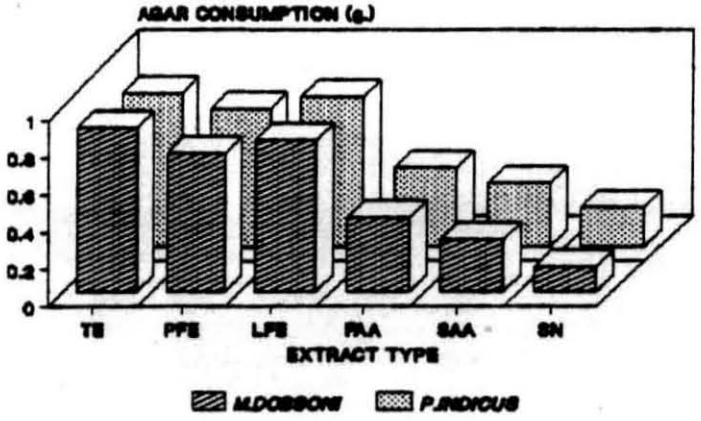
A. FISH EXTRACT



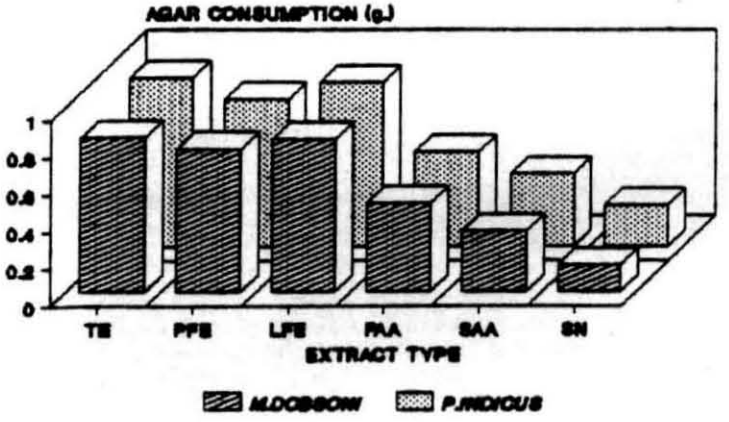
B. CLAM EXTRACT



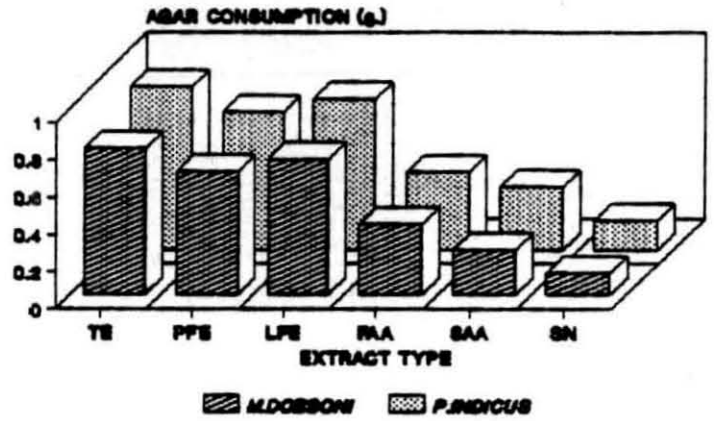
C. SHRIMP EXTRACT



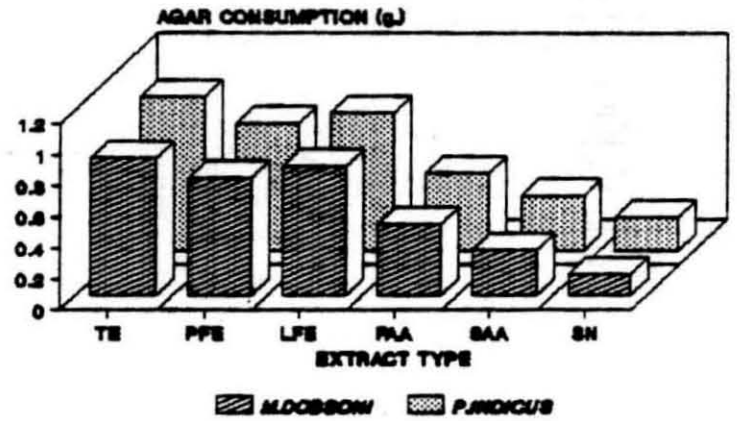
D. CRAB EXTRACT



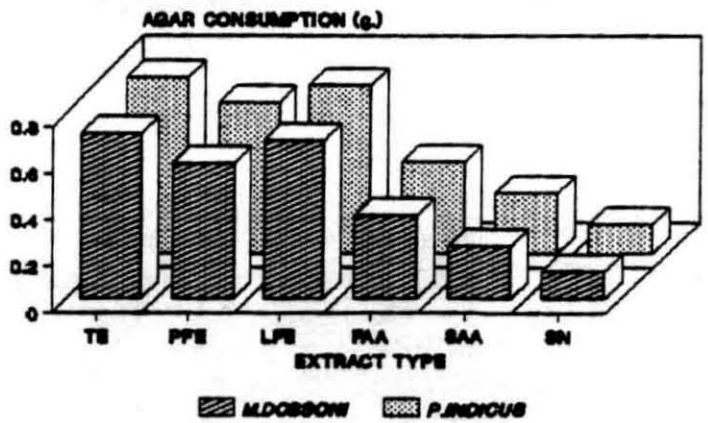
E. OYSTER EXTRACT



F. SQUID EXTRACT



G. MUSSEL EXTRACT



H. SQUILLA EXTRACT

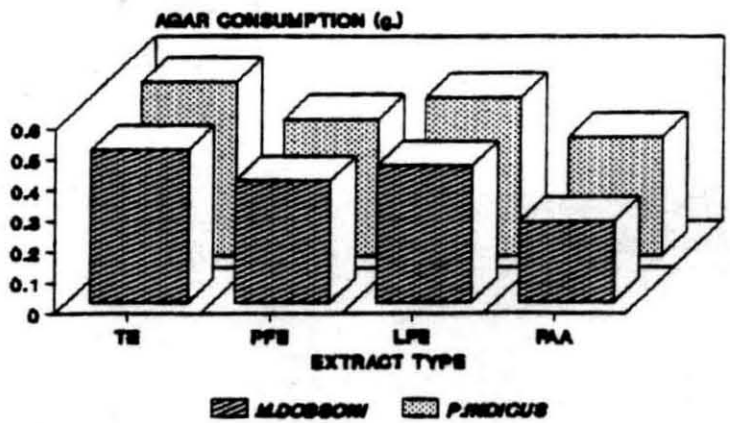
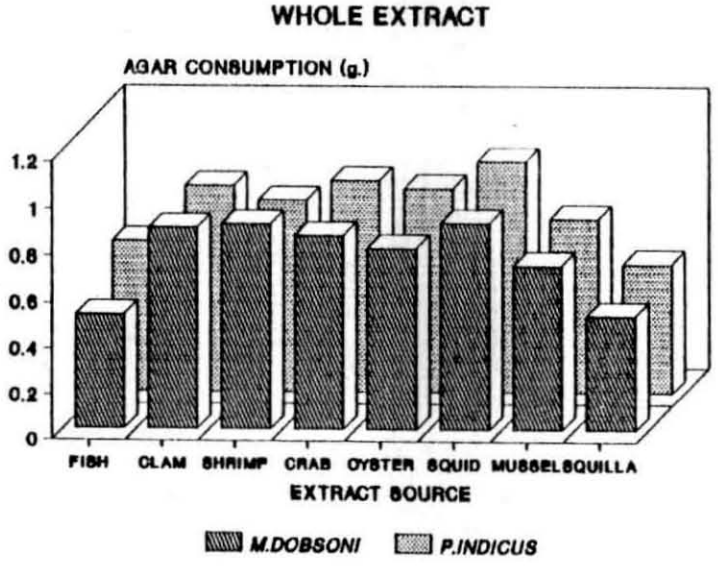
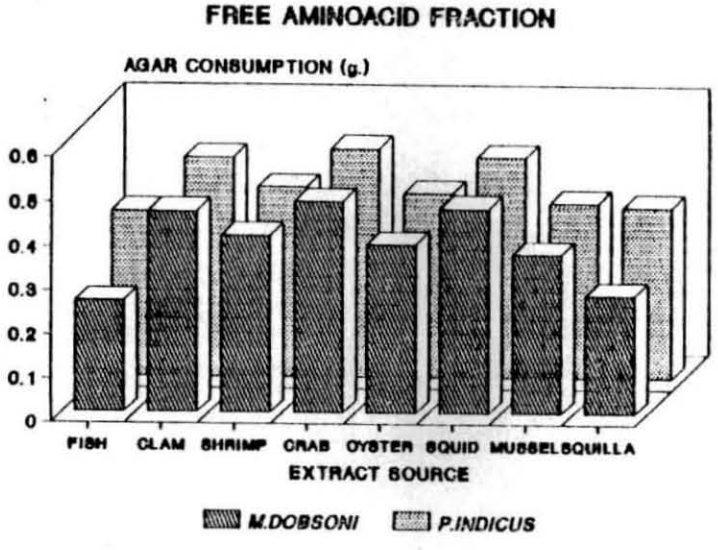


FIG 27 : Agar consumption by P.indicus and M.dobsoni when flavoured with whole extracts and free amino acid fractions from selected tissue sources.





in the agar consumption with a potency loss of 1.3 to 12.3% in P.indicus and 1.9 to 10.6% in M.dobsoni. Minimum potency loss was recorded for lipid free fraction of crab extract in P.indicus and shrimp extract in M.dobsoni, and the maximum for clam and squid extracts in P.indicus and M.dobsoni respectively. Removal of soluble protein from the extract led to a much higher reduction in agar consumption and higher loss in potency than the lipid free fraction. In P.indicus, minimum loss in potency was recorded for protein free fraction of crab extract and maximum for the clam extract, whereas, in M.dobsoni it was for protein free fraction of shrimp and squilla extracts respectively.

When the agar gel was flavoured with free amino acid fraction (FAA) alone, the potency came down to 47.5 to 68.6% in P.indicus and 45.5 to 57.2% in M.dobsoni for various extract types. In both the species maximum consumption occurred when the gel was flavoured with free amino acid fraction of crab, followed by squid, clam and shrimp and minimum for FAA of fish extract. Synthetic amino acid fraction (SAA) produced a potency of 34.9 to 49.5% in M.dobsoni and 27.3 to 39.0% in P.indicus. The SAA fraction based on the amino acid profile of clam produced maximum agar consumption and of fish the minimum consumption in both the species. The potency of synthetic amino acid fraction compared to the free amino acid fraction was low in both species and is of the order of 54% to 86% in P.indicus and 66 to 86% in M.dobsoni.

Flavouring the agar gel with synthetic nucleotide mixture, based on the nucleotide profile of tissue extracts produced considerable variation in the agar consumption ($P<0.05$). In both the species the synthetic nucleotide fraction of crabs elicited maximum agar consumption followed by that of squid and shrimp. The potency of nucloetide fraction was 12.7 to 21.5% in P.indicus and 16.8 to 26.1% in M.dobsoni. The ingestive potency of synthetic nucleotide

TABLE 47 : AGAR GEL CONSUMPTION (g/100 g BODY WEIGHT OF SHRIMP) AND THE RELATIVE INGESTIVE ACTIVITY OF THE TEST SAMPLES FOR P.INDICUS AND M.DOBSONI IN A TWO CHOICE FEEDING PREFERENCE TEST.

STIMULI	P.indicus		M.dobsoni	
	Agar Gel Consumption (g)	Relative Activity	Agar Gel Consumption (g)	Relative Activity
Synthetic amino acid fraction of shrimp (Control)	0.751	100	0.698	100
a. Mixtures				
Neutral amino acids	0.670	89.2	0.634	90.83
Acidic amino acids	0.352	46.87	0.331	47.42
Basic amino acids	0.624	83.09	0.609	87.25
Aromatic amino acids	0.327	43.54	0.327	46.85
b. Nucleotides and Sugars				
Sugars (glucose and sucrose)	0.172	22.90	0.164	23.50
Betaine	0.272	36.22	0.209	29.94
AMP	0.563	74.97	0.521	74.64
ADP	0.208	27.70	0.158	22.64
ATP	0.138	18.38	0.067	9.66
IMP	0.619	82.42	0.602	86.25
Hypoxanthine	0.168	22.37	0.104	14.90
Inosine	0.617	82.16	0.518	74.21

Table 47 (Contd..)

STIMULI	P.indicus		M.dobsoni	
	Agar Gel Consumption (g)	Relative Activity	Agar Gel Consumption (g)	Relative Activity
c. Amino acid				
Synthetic Amino acid Fraction of Shrimp	0.751	100	0.698	100
Alanine	0.366	48.79	0.232	33.24
Arginine	0.449	59.79	0.242	34.67
Aspartic acid	0.418	55.66	0.597	82.52
Glutamic acid	0.327	43.54	0.422	60.46
Glycine	0.325	43.28	0.238	34.10
Histidine	0.339	45.14	0.240	34.38
Isoleucine	0.274	36.48	0.486	69.63
Leucine	0.242	32.20	0.514	73.64
Lysine	0.624	83.09	0.278	39.83
Methionine	0.629	83.12	0.538	77.08
Ornithine	0.209	27.83	0.181	25.93
Phenylalanine	0.239	31.82	0.207	29.60
Proline	0.484	64.45	0.305	43.70
Serine	0.242	32.22	0.218	31.23
Taurine	0.410	54.59	0.401	51.45
Threonine	0.241	32.09	0.271	38.83
Tryptophan	0.277	36.88	0.505	72.35
Tyrosine	0.085	11.32	0.175	25.07
Valine	0.181	24.10	0.034	4.87

Table 47 (c) represents the stimulatory efficacy of different amino acids on agar consumption. Amino acids differ significantly in their ability to induce ingestion activity in shrimps ($P<0.05$). Species also differ significantly in their specificity to different amino acids studied ($P<0.05$). Methionine produced maximum agar consumption and higher relative activity in P.indicus followed by lysine, proline, arginine, aspartic acid, taurine, alanine and histidine in the order. Maximum relative activity was recorded for methionine (83.12%) in P.indicus and minimum for tyrosine (11.32%). In M.dobsoni aspartic acid produced maximum agar consumption with a relative activity of 82.52% and minimum by alanine (4.87%) The order of preference of M.dobsoni towards amino acids were aspartic acid, methionine, leucine, tryptophan, isoleucine, glutamic acid, taurine and proline.

7. EFFECT OF ATTRACTANTS AND STIMULANTS ON FEED INTAKE

The results of the bioassay showed that the feeding stimulant activities of amino acids, nucleotides and related compounds and their combinations varied significantly as depicted from the Daily Feed Intake Ratio and Relative Activity (Tables 48-52), ($P<0.05$). Since the major portion of the feeding stimulant activity of tissue extracts were confined to the amino acid and nucleotides fractions, they were divided into sub-fractions and their feeding stimulant activities were studied individually and in combinations.

The feeding stimulant activity of amino acids in P.indicus and M.dobsoni is presented in table 48 as Daily Feed Intake Ratios (DFIR) and Relative Activity. Amino acids varied significantly in its stimulatory activity and at the same time species also a differ in their preference to amino acids ($P<0.05$). P.indicus showed preference to methionine, lysine, proline, arginine, aspartic acid, taurine and alanine, whereas M.dobsoni preferred

TABLE 48 : FEEDING STIMULANT ACTIVITIES OF AMINO ACIDS IN SYNTHETIC EXTRACTS IN P.INDICUS AND M.DOBSONI

TEST STIMULI	DAILY FEED INTAKE RATIO		RELATIVE ACTIVITY	
	P.indicus	M.dobsoni	P.indicus	M.dobsoni
Squid extract	3.89	3.75	100	100
Alanine	2.37	2.13	60.93	56.80
Arginine	2.66	2.24	68.38	59.73
Aspartic acid	2.59	2.34	66.58	62.40
Glutamic acid	2.31	2.31	59.38	61.60
Glycine	2.24	2.20	57.58	58.67
Histidine	2.34	2.22	60.15	59.20
Isoleucine	2.22	2.32	57.07	61.87
Leucine	2.21	2.33	56.81	62.13
Lysine	2.86	2.29	73.52	61.07
Methionine	2.97	2.33	76.35	62.13
Ornithine	1.79	1.84	46.02	48.20
Phenylalanine	2.19	2.01	56.30	58.67
Proline	2.83	2.30	72.75	61.33
Serine	2.22	2.02	57.07	53.87
Taurine	2.43	2.31	62.47	61.60
Threonine	2.19	2.27	56.30	60.53
Tryptophan	2.23	2.32	57.33	61.87

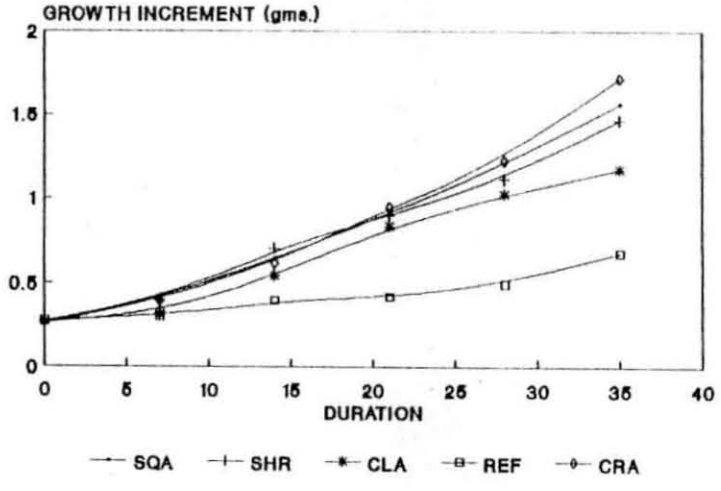
TABLE 49 : FEEDING STIMULANT ACTIVITIES OF NUCLEOTIDES AND RELATED COMPOUNDS IN SYNTHETIC EXTRACTS IN P.INDICUS AND M.DOBSONI

TEST STIMULI	P.indicus		M.dobsoni	
	Daily feed intake Ratio	Relative Activity	Daily Feed intake Ratio	Relative Activity
Squid extract	3.89	100	3.75	100
AMP	2.85	73.26	2.73	72.80
ADP	2.53	65.04	2.50	66.67
IMP	2.90	74.55	2.87	76.53
Inosine	2.72	69.92	2.54	67.73

TABLE 50 : FEEDING STIMULANT ACTIVITIES OF CHEMICAL FRACTIONS OF SYNTHETIC EXTRACTS IN PENAEUS INDICUS AND METAPENAEUS DOBSONI

TEST STIMULI	P.indicus		M.dobsoni	
	Daily feed intake Ratio	Relative Activity	Daily Feed intake Ratio	Relative Activity
Squid tissue extract	3.89	100	3.75	100
Amino acid + nucleotides (Squid) (AS)	3.52	90.49	3.31	88.27
Amino acid + nucleotides (Clam) (AC)	2.81	72.24	2.59	69.07
Amino acid + nucleotides (Shrimp) (ASH)	3.33	85.60	3.02	80.53
Amino acid + nucleotides (Crab) (ACR)	3.62	93.06	3.47	92.53
Betaine	1.97	50.64	2.16	57.6
AS + Betaine	3.74	96.14	3.62	96.53
AC + Betaine	2.92	75.06	2.71	72.27
ASH + Betaine	3.41	87.66	3.27	87.20
ACR + Betaine	3.73	95.89	3.65	97.33

FIG 28 : Effect of supplementation of selected attractant /stimulant mixtures in semi-purified diets on the growth of P.indicus



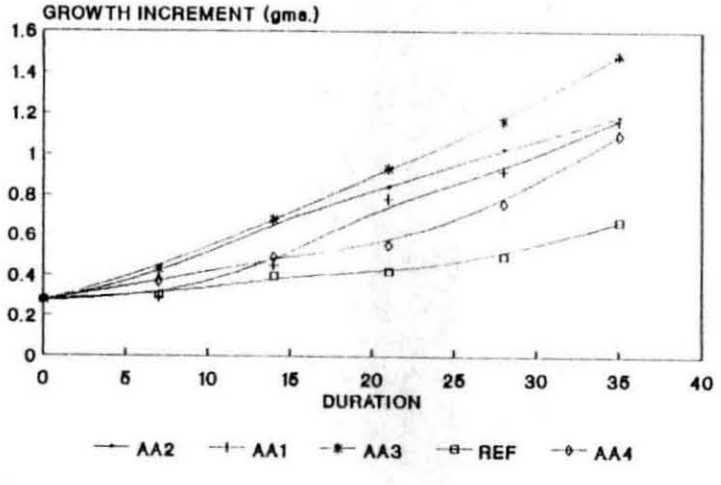


TABLE 51 : FEEDING STIMULANT ACTIVITIES OF AMINO ACID FRACTIONS IN P.INDICUS AND M. DOBSONI

TEST STIMULI	P.indicus		M.dobsoni	
	Daily feed intake Ratio	Relative Activity	Daily Feed intake Ratio	Relative Activity
Squid extract	3.89	100	3.75	10
Acidic amino acids	0.25	6.43	0.10	2.67
Basic amino acids	1.23	31.62	1.19	31.73
Aromatic amino acids	0.12	3.08	0.09	2.40
Neutral amino acids	1.75	44.99	1.59	42.40

Table 52 Contd...

	TEST STIMULI	P.indicus		M.dobsoni	
		Daily Feed Intake Ratio	Relative Activity	Daily Feed Intake Ratio	Relative Activity
13.	L-arginine + L-alanine + Glycine	2.65	68.12	2.31	61.60
14.	L-Proline + Betaine + Glycine + L-alanine	2.57	66.07	2.32	61.87
15.	L-glutamic acid + glycine	2.71	69.67	2.72	72.53
16.	L-tyrosine + L-phenylalanine + L-histidine	2.30	59.13	2.27	60.53
17.	L-tyrosine + L-phenylalanine + L-lysine	2.42	62.21	2.43	64.80
18.	Glycine + L-alanine + L-glutamic acid + Inosine + Betaine	2.86	73.52	2.67	71.20
19.	Glycine	2.23	57.33	2.07	5.52
20.	L-proline + L-alanine +	2.48	68.89	2.69	71.73
21.	Glycine + L-proline + L-alanine	2.91	74.81	2.89	77.07
22.	L-glutamic acid + Glycine + Taurine	2.73	70.18	2.71	72.27
23.	Taurine + Glycine + Arginine + Betaine	2.63	67.61	2.34	62.40

aspartic acid, methionine, leucine, tryptophan, isoleucine, glutamic acid and taurine. Both species showed least preference to ornithine and betaine-HCL. In general the DFI was smaller in M.dobsoni than in P.indicus, whereas the relative activity showed more or less the same trend in both species.

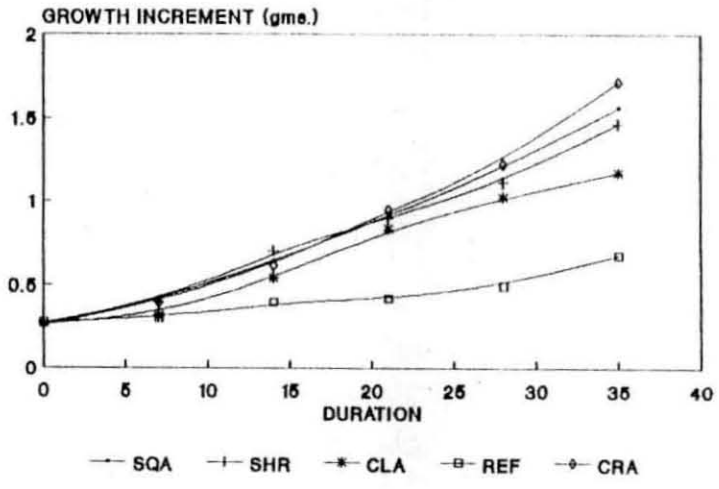
The DFIR and relative activity of nucleotides when incorporated in casein diets are given in Table 49. Nucleotides differed significantly in the feeding stimulant activities ($P<0.05$). Among the nucleotides IMP produced the maximum feeding stimulant property followed by AMP in both species. Both species showed similar preference to nucleotides.

The synthetic tissue extracts differed significantly in their DFIR and relative activities (Table 50) ($P<0.05$). Synthetic crab extract showed the maximum stimulant activity followed by squid and shrimp extract in both species. Betaine which showed poor activity when supplemented alone; significantly increased the stimulant activity of synthetic extracts when supplemented in combinations with them ($P<0.05$).

Table 51 represents the feeding stimulant activities of different amino acid groups. The DFIR and relative activity of these amino acids varied significantly ($P<0.05$). Neutral amino acids followed by basic amino acids produced the maximum feeding stimulant activity. About 40 to 50% of the activity resided in the neutral amino acid group for both species. Aromatic amino acids contributed minimal feeding stimulant activity.

The DFIR and relative activity of amino acid mixtures in P.indicus and M.dobsoni are given in Table 52. Different combinations varied significantly in their activity ($P<0.05$). In both species L-methionine, L-arginine L-alanine, L-proline and Inosine (1:1:1:1) combination produced the highest

FIG 28 : Effect of supplementation of selected attractant /stimulant mixtures in semi-purified diets on the growth of P.indicus



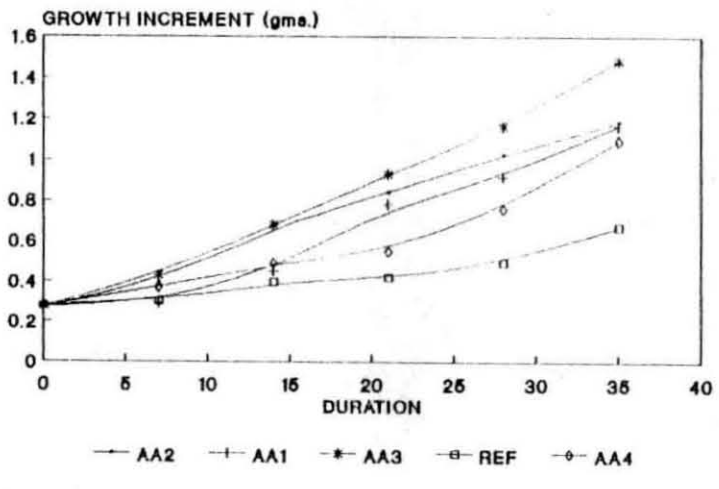


Table 52 Contd...

	TEST STIMULI	*P.indicus*		*M.dobsoni*	
		Daily Feed Intake Ratio	Relative Activity	Daily Feed Intake Ratio	Relative Activity
13.	L-arginine + L-alanine + Glycine	2.65	68.12	2.31	61.60
14.	L-Proline + Betaine + Glycine + L-alanine	2.57	66.07	2.32	61.87
15.	L-glutamic acid + glycine	2.71	69.67	2.72	72.53
16.	L-tyrosine + L-phenylalanine + L-histidine	2.30	59.13	2.27	60.53
17.	L-tyrosine + L-phenylalanine + L-lysine	2.42	62.21	2.43	64.80
18.	Glycine + L-alanine + L-glutamic acid + Inosine + Betaine	2.86	73.52	2.67	71.20
19.	Glycine	2.23	57.33	2.07	5.52
20.	L-proline + L-alanine +	2.48	68.89	2.69	71.73
21.	Glycine + L-proline + L-alanine	2.91	74.81	2.89	77.07
22.	L-glutamic acid + Glycine + Taurine	2.73	70.18	2.71	72.27
23.	Taurine + Glycine + Arginine + Betaine	2.63	67.61	2.34	62.40

TABLE 51 : FEEDING STIMULANT ACTIVITIES OF AMINO ACID FRACTIONS IN P.INDICUS AND M. DOBSONI

TEST STIMULI	P.indicus		M.dobsoni	
	Daily feed intake Ratio	Relative Activity	Daily Feed intake Ratio	Relative Activity
Squid extract	3.89	100	3.75	10
Acidic amino acids	0.25	6.43	0.10	2.67
Basic amino acids	1.23	31.62	1.19	31.73
Aromatic amino acids	0.12	3.08	0.09	2.40
Neutral amino acids	1.75	44.99	1.59	42.40

TABLE 52 : FEEDING STIMULANT ACTIVITIES OF SOME AMINO ACID COMBINATIONS IN P.INDICUS AND M.DOBSONI

	TEST STIMULI	P.indicus		M.dobsoni	
		Daily Feed Intake Ratio	Relative Activity	Daily Feed Intake Ratio	Relative Activity
1.	Squid extract	3.89	100	3.75	100
2.	L-alanine + L-methionine + L-serine + L-proline	2.61	67.10	2.62	69.87
3.	Inosine	2.95	75.84	2.91	77.60
4.	L-arginine + L-alanine + L-proline + L-methionine	2.56	65.81	2.53	67.47
5.	(4) + Inosine	3.05	78.41	3.01	80.27
6.	Inosine + Glycine	2.93	75.32	2.61	69.60
7.	Glycine + L-proline + Betaine + L-alanine	2.62	67.35	2.49	66.40
8.	L-proline + Glycine	2.34	60.15	2.21	58.93
9.	L-alanine + Glycine + L-arginine + L-serine	2.72	69.92	2.74	73.07
10.	L-proline + L-alanine + L-leucine	2.26	58.10	2.28	60.80
11.	Glycine + L-phenylalanine + L-lysine + L-methionine	2.73	70.18	2.71	72.27
12.	L-arginine + L-methionine + L-proline	2.92	75.06	2.90	77.33

aspartic acid, methionine, leucine, tryptophan, isoleucine, glutamic acid and taurine. Both species showed least preference to ornithine and betaine-HCL. In general the DFI was smaller in M.dobsoni than in P.indicus, whereas the relative activity showed more or less the same trend in both species.

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The DFIR and relative activity of amino acid mixtures in P.indicus and M.dobsoni are given in Table 52. Different combinations varied significantly in their activity ($P<0.05$). In both species L-methionine, L-arginine L-alanine, L-proline and Inosine (1:1:1:1) combination produced the highest

activity followed by L-alanine, L-methionine, L-serine, L-proline and Inosine (1:1:1:1:1) combinations. When inosine was removed from the mixtures, feed intake and relative activity decreased considerably. Hence it can be concluded that inosine produces a synergistic effect. Glycine (Table 52) and betaine (Table 50) also exhibited synergistic effects on the feeding stimulant activity when supplemented along with other compounds. These compounds when supplemented singly exhinited poor activity, but on supplementing with other compounds in combination significantly increased the DFI and relative activity ($P<0.05$).

8. EFFECT OF FEEDING STIMULANTS ON GROWTH PERFORMANCE

8.1 LABORATORY TRIALS

8.1.1. **Study with semi-purified diets:** Dietary supplementation of various feeding stimulants significantly increased the Daily Feed Intake (DFI), Feed Assimilation Efficiency (FAE), Specific Growth Rate (SGR), weight gain and survival with better Food Conversion Ratio (FCR) compared to unflavoured control (Table 53) ($P<0.05$). Efficacy of the feeding stimulants to induce feed intake varies considerably with the stimulant combination. Growth of the shrimps were faster for flavoured diets, than for the control diet, from the initial stage of rearing (Fig. 28).

Supplementing synthetic free amino acid fraction of tissue extracts (SAA) in casein diets significantly ($P<0.05$) increased the feed intake and improved the growth performance compared to control (Table 53a). The DFI of flavoured diets increased from 0.090g of control to 0.113 and 0.124g for flavoured diets. FAE was also high for flavoured diets (81.36 to 85.29%) than the control (72.26%). FCR of the flavoured diets were ranging between 2.93 and

TABLE 53: FEED SELECTIVITY AND GROWTH PERFORMANCE OF P. INDICUS FLAVOURED WITH FEEDING STIMULANTS IN A 35 DAY GROWTH TRIAL.

DIET TYPE (**)	WEIGHT GAIN (g)	DFI*	FAE (%)	SURVIVAL (%)	FCR	SGR
a. Semipurified diets						
REF	0.394	0.090	72.26	45.0	3.81	0.025
SQA	1.294	0.122	83.38	82.0	3.02	0.05
SHA	1.188	0.117	82.78	74.0	2.99	0.048
CLA	0.908	0.113	81.36	60.0	3.12	0.043
CRA	1.451	0.124	85.29	62.0	2.93	0.053
AA_1	0.897	0.118	82.68	78.0	3.34	0.041
AA_2	0.916	0.117	81.90	81.0	3.28	0.092
AA_3	1.209	0.123	78.92	73.5	3.24	0.048
AA_4	0.821	0.116	77.20	64.0	3.41	0.039
b. Compounded diets						
FS_1	2.546	0.129	84.46	98.0	2.78	0.068
FS_2	3.248	0.132	89.25	100.0	2.69	0.073
FS_3	3.121	0.131	86.5	100.0	2.70	0.072
FF_1	1.769	0.126	84.6	90.0	2.97	0.057
FF_2	2.383	1.130	85.73	100.0	2.83	0.065
FF_3	2.154	0.128	85.14	98.0	2.89	0.062
FC_1	1.959	0.123	84.58	88.0	3.09	0.060
FC_2	2.566	0.129	88.45	97.0	2.76	0.066
FC_3	2.304	0.128	86.43	100.0	2.81	0.063
FD_1	2.760	0.130	87.47	100.0	2.71	0.069
FD_2	3.376	0.134	90.32	100.0	2.66	0.075
GD_3	3.272	0.132	88.95	98.0	2.68	0.074

* gm feed/gm body wt of shrimp/day

** Refer Table 6 and 8a for detail.

3.12, whereas for the control it was 3.81. SGR was also high for the flavoured diets (0.043 to 0.053), than the unflavoured casein diet (0.025). During the 35 days feeding trial a weight increment of 0.908 to 1.451g was obtained for flavoured diets and 0.394g for control diet. Survival rate of the shrimps was increased to 60 to 82% for the flavoured diets from 45% of the unflavoured diet. SAA based on different tissue sources significantly differed in their ability to induce feed intake and to produce growth ($P<0.05$). SAA of crab (CRA) produced the maximum feed intake and growth followed by that of squid (SQA) and shrimp (SHA). DFI, FAE and SGR was high for CRA and SQA. But FCR was better for CRA (2.93), SHA (2.99) and SQA (3.02) in the order.

Various amino acid mixtures and taurine as feeding stimulants significantly improved the feed intake, FAE, FCR and growth of the shrimp ($P<0.01$). DFI for the diets flavoured with amino acid mixture was 0.116 to 0.123 and FAE was between 78.92 to 82.68% and for taurine it was 0.116 and 77.2% respectively. FCR for the stimulant incorporated diet was small, (3.24 to 3.34) for amino acid mixture and 3.41 for taurin. Stimulant incorporated diets, produced a weight increment of 0.897 to 1.209 for various amino acid mixtures, and 0.821 for taurine, incorporated diet. All these values for flavoured diets are lying well above that of the control diet (REF). Test diet AA_3 incorporated with methionine arginine, alanine, proline, and inosine performed better in terms of DFI, SGR, FCR and weight gain. But FAE was high for AA_1, with glutamic acid, glycine, taurine and betaine where as survival was high for AA_2 with serine, methionine, alanine, proline and inosine.

Flavouring the diets with taurine (AA_4) increased the feed intake, FAE, SGR and doubled the weight increment with small FCR than the control. But its performance was poor compared to the amino acid mixtures tested.

SAA is performing better in terms of food intake, FAE, FCR, and growth performance than the L-amino acid mixtures (AA).

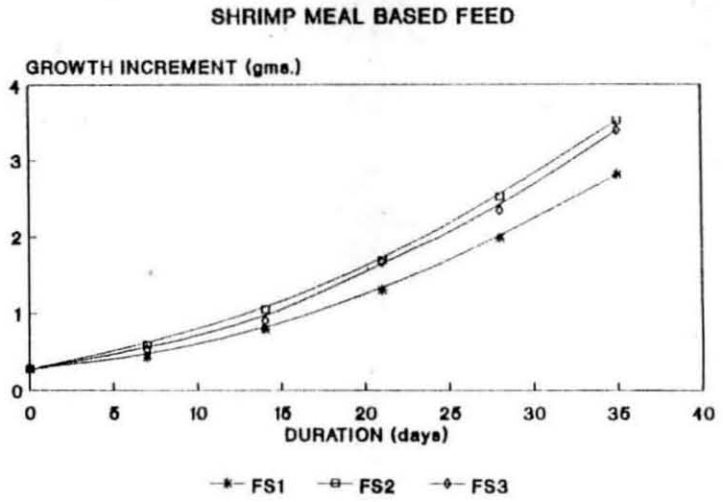
8.1.2. **Study with compounded diets:** Compounded diets with crude animal meals as protein bases were preferred by shrimps than semipurified diets, as has been evident from the improved DFI, FAE, FCR, SGR and weight gain for the former (Table 53b, Fig 29 (a-d). Supplementing selected feeding stimulants in compounded diets, improved its palatability and growth efficacy. DFI, FAE and SGR increased and FCR, improved significantly for the flavoured diets than the corresponding control ($P<0.05$). Stimulant mixture based on the free amino acid profile of crab tissue extract performed better with high values for DFI, FAE, SGR and growth than the methionine, arginine, alanine, serine, proline and inosine mixture, when supplemented at the same level.

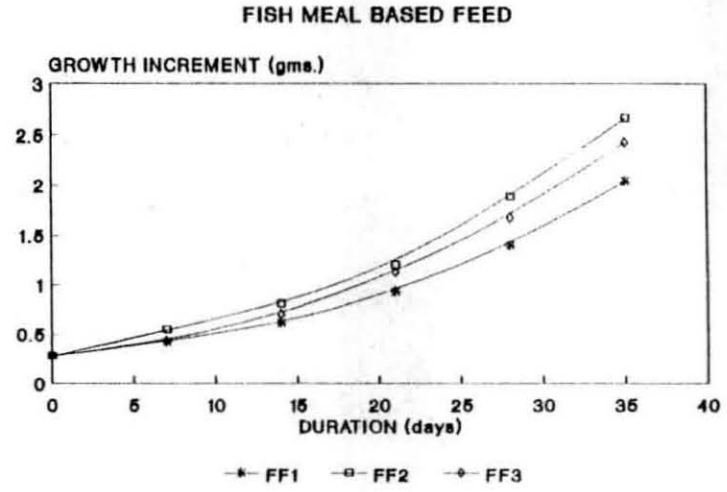
Among the single meal based diets FAE, DFI, SGR and growth was high for shrimp meal based diets with small FCR followed by that of clam and fish meal based diets. Weight gain for the shrimps fed with shrimp meal based diet was 2.546 g for control and 3.248 and 3.121 for the flavoured diets during the 35 day rearing trial. But the standard diet with mixed protein base performed better than the single meal based diets on feed consumption and growth performance. DFI, FAE SGR and growth was higher for the standard diets than the corresponding values for the single meal based diet. Feed intake and growth performance of the unflavoured standard diet was higher than the flavoured clam and fish meal based diet, but was slightly less than the flavoured shrimp meal based diets.

8.2 FIELD TRIAL

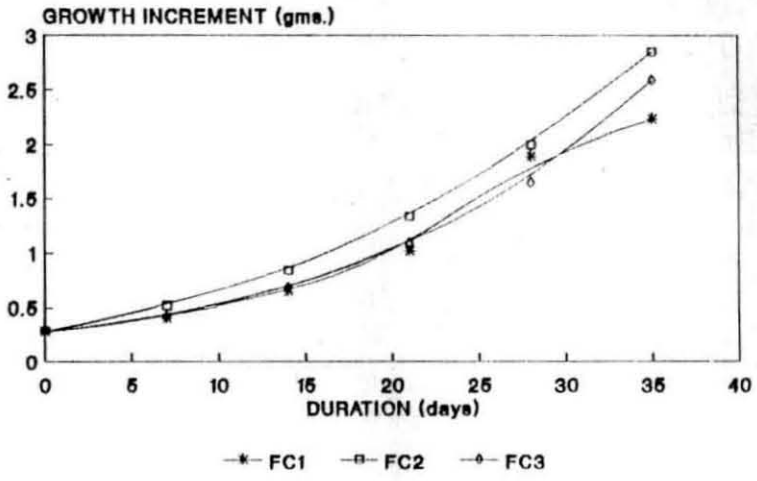
Results of the multiple choice feeding trial under field condition is given in table 54. Incorporation of flavourant compounds in the feed

FIG 29 : Effect of supplementation of selected attractant/stimulant mixtures in compounded diets on the growth of P.indicus





CLAM MEAL BASED FEED



STANDARD FEED

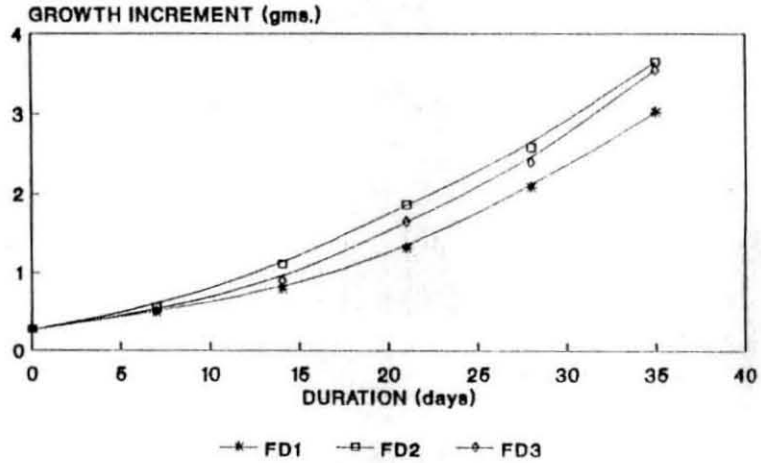


TABLE 54 : SELECTIVITY OF P.INDICUS TO TEST DIETS INCORPORATED WITH DIFFERENT FLAVOURANT COMPOUNDS UNDER FIELD CONDITIONS

FEED TYPE **	FEED CONSUMED(g)	FORAGING RATIO	SELECTIVITY INDEX	FEED TYPE	FEED CONSUMED(g)	FORAGING RATIO	SELECTIVITY INDEX
F_1	17.918	0.523	-0.313	F_{17}	17.591	0.513	-0.322
F_2	46.06	1.34	0.145	F_{18}	10.562	0.308	-0.529
F_3	29.98	0.86	-0.062	F_{19}	30.215	0.882	-0.063
F_4	30.277	0.884	-0.064	F_{20}	15.781	0.460	-0.369
F_5	75.118	2.192	0.373	F_{21}	40.315	1.176	0.081
F_6	64.970	1.905	0.312	F_{22}	12.578	0.367	-0.463
F_7	60.210	1.757	0.275	F_{23}	20.210	0.589	-0.258
F_8	40.790	1.190	0.087	F_{24}	75.550	2.20	0.376
F_9	45.292	1.322	0.139	F_{25}	80.752	2.356	0.404
F_{10}	68.550	1.959	0.336	F_{26}	40.262	1.175	0.080
F_{11}	47.510	1.386	0.162	F_{27}	10.359	0.302	-0.536
F_{12}	50.250	1.466	0.189	F_{28}	22.610	0.658	-0.205
F_{13}	40.770	1.190	0.087	F_{29}	32.591	0.951	-0.025
F_{14}	10.672	0.311	-0.525	F_{30}	5.271	0.154	-0.834
F_{15}	10.261	0.299	-0.539	F_{31}*	5.425	0.158	-0.725
F_{16}	2.230	0.062	-0.940	*-Control			

** Refer Table 9 for detail.

significantly improved the feed intake compared to control ($P<0.05$). The foraging ratio (FR) and index of selectivity (SI) varied significantly for different feed types incorporated with flavourants ($P<0.01$). Feed types with a foraging ratio above 1 and a positive value for index of selectivity above zero indicate preference for that feed type compared to average feed consumption, where as a forage value below 1 and a negative value for index of selectivity represents poor preference and/or avoidance.

Incorporation of the flavourant compounds in the feed increased the feed consumption by P.indicus in grow out ponds (10.261 to 80.752g) compared to unflavoured control (5.425g.) except for feed type F_{16} (2.23g) incorporated with histidine, arginine, proline and betaine and F_{30} (5.271g) incorporated with taurine and lysine. The foraging ratio and index of selectivity was also the lowest for this feed types and is of the order of 0.062 and -0.94 and 0.154 and -0.834 for F_{16} and F_{30} respectively. The same for control diet was 0.158 and -0.725 indicating poor preference. Maximum feed consumption (80.752g) was recorded for F_{25} incorporated with arginine, alanine, proline, inosine and methionine, followed by F_{24} (75.55g) with alanine, methionine, serine, proline and inosine. The foraging ratio and index of selectivity was also high for F_{25} (2.356 and 0.404 respectively) and F_{24} (2.2 and 0.376), compared to other amino acids and their combinations.

When the flavourant compounds were supplemented individually methionine (F_5) produced maximum feed consumption (75.118) with highest foraging ratio (2.192) and index of selectivity (0.373), followed by lysine (F_{10}) with a feed consumption of 68.55 g. and with 1.959 and 0.336 as the foraging ratio and index of selectivity respectively. Whereas aspartic acid (F_1) produced the lowest food consumption associated with small values of foraging ratio (1.19)

and index of selectivity (0.087). The order of preference of amino acids based on the feed selectivity was methionine, lysine, glycine, betaine, serine, alanine, proline, arginine, taurine and aspartic acid.

Selected amino acids and their combinations when supplemented in compounded feeds increased the food consumption by P.indicus under field conditions compared to control. Many amino acids had synergistic effect and significantly increased the feed consumption with high foraging ratio and selectivity index, than when they are supplemented individually ($P<0.05$). Some combinations when supplemented, the feed consumption decreased sharply with poor foraging ratio than its constituent amino acids. Feed types F_{16} and F_{30} produced poor feed intake than the unflavoured control. Poor feed consumption was also observed for feed types F_{18}, F_{22} and F_{27}. But their constituent amino acids when supplemented individually produced better feed consumption with large foraging ratios and high index of selectivity.

DISCUSSION

1. TESTING METHODOLOGY

All animals selected for observations were at the intermoult stage of the cycle. The particular stage was selected to avoid variation in responsiveness likely to arise from the physiological state of the animals. Harpaz et.al. (1987a) stated that the responsiveness of the animals to chemical stimuli varies with the moult cycle, being most responsive at the intermoult stage and least at post moult stage.

Behavioural, physiological and electrophysiological responses have been employed in studying the chemoreceptiveness of decapods. Behavioural responses related to chemoreception and feeding in decapods include flicking of antennules, walking or swimming, grasping and lifting movements of pereopods and movements of mouth appendages. The only physiological responses used so far to indicate chemoreception have been alteration in heart rate, induced by chemoreceptors in the branchial chamber (Zimmer-Faust et.al., 1979), but the relation between this response and behaviour have not been firmly established. In electrophysiological studies impulses from nerve bundles of chemoreceptors are recorded when the receptors are exposed to test solutions. However the result can only indicate the possibility of detection and doesnot allow behavioural prediction.

In the present study only behavioural responses have been used to evaluate chemoreception in P.indicus and M.dobsoni. The behavioural descriptors, described by Costero and Meyers (1993) for P.vannamei to chemical feeding stimuti was used as a base line for the present study. In this study

the behavioural responses of the shrimps were evaluated both qualitatively and quantitatively using the bioassay systems and the activity recorder described earlier.

1.1 BIOASSAY SYSTEM

One of the main prerequisite in the evaluation of chemotactic responses of intact animals is a reliable means of bioassay. The bioassay system developed for the present study can be used for evaluating the behavioural responses of all groups of aquatic animals especially crustaceans, irrespective of their size and age, towards different chemical stimuli. This system is a modification of the bioassay system developed by Harada et.al. (1986) for oriental weather fish and includes all the salient features of the assay systems described by Mcleese (1970), Mackie and Shelton (1972), Hindley (1975), Devine and Atema (1982) and Costero and Meyers (1993).

The systems developed by McLese (1970) and Mackie and Shelton (1972) are suited mainly for work with large decapod crustaceans, whereas in the case of other systems, the stimuli may get accumulated in the system itself to some extent. In the present system all these defects were very effectively eliminated. Moreover the present bioassay procedure is very simple, convenient and trouble free. This allows the investigator to quickly recognise response inducing stimuli and to compare in a qualitative and quantitative manner the relative potency of closely related chemicals.

1.2 ACTIVITY RECORDER

Fish activity recorder was used for the quantitative evaluation of behavioural responses of shrimps to chemical stimuli. This instrument picks

up even the minute movements of the appendages and these are recorded. This system is simple, portable and remote control type and can be used for continuous recording with least disturbance to the best animals, and the observations are free from the observer's influence. Another advantage of the system is that; the feeding movements in responses to feeding stimuli can be easily distinguished from that caused due to stress or disturbance.

2. CHEMOTACTIC BEHAVIOUR OF SHRIMPS

2.1 CHEMOTACTIC INDICES

To study the chemotactic property of various natural and artificial stimuli; attractant and repellent indices were developed using squid tissue extract as attractant sample and squid ink as repellent sample. Many workers have also used different tissue extracts as attractant sources for evaluating the chemotactic behaviour of shrimps (Carr et.al., 1974, Carr, 1978), lobsters (Mackie and Shelton, 1972, Mackie, 1973; Mcleese, 1973 a,b) and crabs (Pearson and Olla, 1977). Squid ink when introduced into the medium, P.indicus and M.dobsoni exhibited typical repellent and feeding inhibiting behaviour. A similar feeding inhibitant activity was reported by Kittredge et.al. (1974) in the lobster towards octopus ink and was reported to be due to the presence of dopachrome present in the ink.

A chemotactic index, with special reference to attraction and repellence was developed for both P.indicus and M.dobsoni from the percentage response of animals using linear regression procedures. Two indices were derived from the co-efficient as difference (Db values) and ratio (Rb Values) between sample and control co-efficients. The Db values were always above 'zero' and increased with attractant concentration, whereas in the case of repellent it

was below zero and decreased with increasing concentration (Fig 4). The Rb values for the attractant were above "1" and increased with the attractant concentration (Fig 5a). In the case of repellent sample the Rb values were always below "1" and decreased with increasing repellent concentration (Fig 5b). A "zero" Db value and an Rb value "1" indicates neutral activity. It was assumed that materials showing less of an attractant effect than that of the control was repellent.

To ascertain the validity of the chemotactic indices, the difference and ratios of co-efficients were investigated in further studies using different tissue extracts and their fractions. (Table 15 a,b). Similar indices were developed by Harada et.al. (1986) for fishes and shellfihes. Conclusively these indices were much simple, are easy to evaluate and provided with the advantage of determining the attraction and repellence of samples simultaneously.

2.2 RESPONSE OF SHRIMPS TO ATTRACTANTS AND REPELLENTS

Both P.indicus and M.dobsoni showed no response when sea water was injected into the system. With the increase in concentration of the attractant sample the percentage response increased (Table 10) and the latency to elicit feeding behaviour decreased significantly (Table 12). Antennular flicking, grooming and food picking behaviour increased with response to increasing attractant concentration. A similar increase in the feeding response was reported in the crabs, Callinectes sapidus (Pearson and Olla, 1977; 1979) and in Cancer magister (Pearson et.al., 1979) when exposed to clam extract. Mackie and Shelton (1972) and Fuzessery and Childress (1975) reported increased antennular flicking in several crustaceans as an indication of awareness to sapid solutions.

When exposed to repellent the feeding response of the shrimps decreased rapidly (Table 11) and the time lag to elicit feeding response increased sharply (Table 13), with the increase in concentration of repellent. Shrimps initially exhibited some flicking and grooming movements, but at higher concentration all the feeding responses ceased and were found to move away from the water current which carried the repellent. Similar avoidance behaviour to repellent samples were reported in other crustaccans like Panulirus argus (Kittredge et.al., 1974) in response to octopus ink and in P.interruptus (Zimmer-Faust and Case, 1982b) in response to shrimp cephalothorax extract.

In the case of attractant sample shrimps moved against the current carrying the attractant, towards the sources, and were found concentrating around the delivery tube. These observations concluded that antennular flicking movements increases in the presence of sapid chemicals and may be involved in the orientation towards the stimulus sources. Fuzessery (1978) and Pearson et.al.(1979) also stated that antennular flicking aids in orientation in crustaceans.

The quantitative recording made in the Activity Recorder (Table 14, Fig 6 and 7) confirmed the above findings of increase in the behavioural response with increase in concentration of attractant and decrease in response with repellent concentration.

2.3 CHEMOTACTIC PROPERTY OF TISSUE EXTRACT

The tissue extracts differed significantly ($P<0.01$) in their property to elicit feeding responses in both species. Moreover, P.indicus and M.dobsoni also differed with each other in their preference to different tissue

extracts. Among the tissue extracts tested maximum response was obtained for squid, followed by crab extract and the least response for squilla extract in P.indicus (Fig 8). In M.dobsoni the maximum response was obtained for shrimp extract followed by squid and crab extract and the least for fish extract (Fig 9). Similar studies by different workers have pointed out the most stimulatory natural extracts for various crustacean species. Squid extract was found to be most stimulatory for Homarus gammarus (Mackie and Shelton, 1972) and other crustaceans (Mackie, 1973), crab extract for Palaemonets pugio and other crustaceans (Carr. et.al., 1984) Homarus americanus (Carr and Steele, 1982), shrimp extract for H. americanus (Mcleese, 1978) and Panulirus argus (Daniel and Derby 1988), shrimp head offal for Penaeus vannamei (Holland and Borski, 1993). mussel extract for Penaeus monodon post-larvae (Murai et.al., 1981) cod and lobster extract for H.americanus (Mcleese, 1978) and clam and marine worm for Penaeus japonicus (Konosu et.al., 1966).

Analysis of the tissue extracts has shown that the total free amino acid content and the composition of individual amino acids in each tissue extract varies widely (Table 1 and 4). There is also considerable variation in the relative composition of other extract components like nucleotides (Table 5). Hence from the present study it is very difficult to say which amino acid(s) or amino acid mixtures contribute maximum to the total attractability of the extract. There may be different mixtures of, tertiary amines and other compounds which are highly attractive to shrimps. A similar conclusion has been arrived at by Mackie (1973) while studying the chemical basis of food detection in the lobser, H.gammarus. The highest chemotactic property observed in this study for squid, crab and shrimp extract may be due to the presence of higher total free amino acid content in these tissue extracts.

The difference in the response of P.indicus and M.dobsoni to different natural extracts is due to the fact that their chemoreceptor organ may be tuned to the free amino acids and other nitrogenous bases present in their potential food organisms and that they will respond more strongly to those extracts that are more identical in them amino acid profile with their potential prey. Similar species specific variation has also been reported by several others in crustaceans toward natural and chemical stimuli (Hazlett, 1971 a; Roberston, 1980; Roberston et.al., 1981, Trott and Roberston, 1984; Tierney and Atema, 1988). Nakamura (1987) stated that the chemical sensitivity in different decapod species varies as each species has proper receptors for specific stimuli. Where as Roberston et.al., (1981) suggested that the difference in the specificity of species to different chemical stimuli resulted with the animal's adaptation to different food-limited environment. This conclusion is closer to the conclusion arising from the present study in P.indicus and M.dobsoni.

Among the extract types tested whole extracts gave the maximum response. The chemotactic property of the extract decreased, when different fractions were removed from the whole extract. Present results showed clearly that, amino acids, proteins, nucleotides and a small fraction by the lipid components are responsible for the total attractant property of tissue extracts. But many workers in this line of study reported that feeding responses of the extracts were contributed by the less than Ca.1000 molecular weight component represented by amino acids and related compounds in crustaceans (Laverack, 1963., Case, 1964, Kay 1971, Carr, 1978; Carr et.al., 1978, Johnson and Ache, 1978; Ache, 1982; Derby and Atema 1982 a; b; Trott and Roberston 1984), and that the feeding response can be duplicated either completely or in a larger part with synthetic mixtures of L-amino acids and

betaines. Johnson and Ache (1978) stated that the shrimps feed on different organisms, having major amino acid pools, and that these aminoacids act as excellent feeding stimulants in aquatic environments.

But many have stated that the attractive property of natural extracts could not be accounted for entirely by free amino acid content but rather other components also provide attractability to the extracts (Shelton and Mackie, 1971; Mackie and Shelton, 1992; Mackie, 1973; Carr and Gurin, 1975). These and the present findings indicated that in addition to amino acids there are other components present in the extracts responsible for eliciting feeding responses in crustaceans. Findings of Ache et.al., (1976) supported these results which stated that major stimulants in the aqueous extracts of potential food organism for Panulirus argus were substances of <10,000 M.W. fractions which includes, proteins, peptides, and nucleotides in addition to amino acids and related compounds.

The contribution of chemotactic activity by the free amino acid fraction alone is 78.2% (ranging between 49.59% and 88.78%) for P.indicus and 50.28% (ranging between 38.9 and 64.35%) for M. dobsoni Whereas protein fraction contributed 12.8% of other chemotactic property of the extracts in P.indicus and 17.29% in M. dobsoni and the lipid fraction 3.0% and 6.75% respectively (Table 21). This indicates that in the natural extracts, free amino acids and related compounds form the major attractant and stimulant components. Similar results were also reported by several workers in crustaceans (Case and Gwilliam, 1961; Mackie and Shelton, 1972; Ache et.al., 1976; Johnson and Ache, 1978). Johnson and Ache (1978) reported that the aminoacid component of the shrimp extract account for 60% of the total stimulatory capacity of the extract in lobster, P.argus. Carr (1978) evaluated the potency of various

extract fractions of crab, oyster, mullet and sea urchin in P.pugio, and found that substance of low molecular weight provide 60-100% of the activity of crabs, oyster and sea urchin and 30% of the mullet extract.

However Carr and Gurin (1975) reported that substances of higher molecular weight were primarily responsible for the attractiveness of oyster mantle fluid, human serum and clam extracts to P.pugio. The higher molecular weight fraction of human serum containing mostly proteins and glucose were as attractive as whole serum, while the < 1000 M.W. fraction was less than 20% as active. Similar positive feeding stimulant property of proteins and peptides in decapods were reported by several workers (Carr et.al., 1975; Sick and Beaty 1975; Ache et.al., 1976; Sick, 1976; Hartman and Hartman, 1977; Zimmer Faust and Michel, 1980; Roberston et.al., 1981; Holland and Borski, 1993). Whereas Case (1964), and Trott and Roberston (1984) stated that proteins and peptides were least responsive for crabs. But according to Carr (1978) proteins and other substances with molecular weight greater than Ca 1000 have virtually no contribution to the activity of the total extract in P.pugio.

The present study has shown that lipid fractions of the tissue extract have some, role in the chemotactic property of whole extract, but very low when compared to other components. Bryant et.al., (1989) also made similar observations in Palaemon elegans, that fish oils and other oils rich in highly unsaturated fatty acids alone act as feeding stimulant. But Mackie and Shelton (1972) stated that in H.gammarus oils and fats are not involved in chemostimulation.

The activity of the whole extract in most of the cases were higher than that of the total activity produced when the components of the extract were

provided individually at the same concentration as present in the whole extract. This increased activity of the whole extract may be due to the synergistic effects of the extract component when they act together.

The effective concentration (EC 50 values) required to elicit feeding behaviour in P. indicus and M. dobsoni for whole extract and extract fractions are given in Table 16 (a & b). Linear regression procedure was used to estimate threshold as an approximation of relationship between the percentage of shrimps detecting the presence of stimulus in a given time and the concentration of the stimulus. Comparisons of threshold with those of other crustaceans is difficult because the response criteria, sapid substances, potential food of test animals and food depreviation schedule vary among investigations. Several workers have estimated the effective concentration of extract preparations required to elicit feeding response in crsustaceans like, Cancer magister (Pearson et.al; 1979), Callinectes sapidus (Pearson and Olla, 1977), Panulirus interruptus (Zimmer-Faust and Case, 1983) H.americanus (Mc Leese, 1973b) and H.gammarus (Mackie and Shelton, 1972; Mackie, 1973). The general picture that emerges from combining the findings of several behavioural bioassay in decapods is that extracts of potential food organisms elicit arousal at less than picogram quantities of dry tissue per litre, walking and or searching at microgram quantities, and food handling and/or ingestion at milligram quantities (Mcleese, 1973, Pearson and Olla, 1973). The differences in the detection threshold which was observed between P.indicus and M.dobsoni and those reported by several workers may be due to ecological and evolutionary differences, difference in the morphology and feeding movements and adaptation to various food habits of difference crustacean species.

It was also observed that on removing different components from the whole extract, the EC 50 values increased significantly for both species (Table 16 a & b). These variations in the EC 50 values indicates the relative role of each tissue component in the chemotactic property of tissue extracts. Carr (1978) also observed a similar increase in the dosage required to elicit feeding behaviour in the shrimp P.pugio, when different fractions were tested separately compared to whole extract.

Similarly, on removing the extract fractions the time lag to elicit feeding behaviour (Et 50) also increased significantly (Tables 17, 18, 19). This increase in the time lag was in full agreement with that of the increase in EC 50 values for both species. The chemotactic indices, derived from the response of shrimps to different extracts are given in Table 15 (a & b). The index values for different fractions showed that the chemotactic property of the extract decreased with the removal of its components. The behavioural responses and the unit activities recorded using activity recorder also showed a similar trend (Fig 12 and 13 and Table 22). Moreover, the behavioural responses of the shrimps to stimuli decreased significantly with the removal of different extract fractions. As has been stated above, all these findings also clearly indicate that free aminoacid fraction followed by protein fraction contributes maximum to the chemotactic property of tissue extracts.

3. CHEMOTACTIC PROPERTY OF SYNTHETIC STIMULI

3.1 AMINO ACIDS

Study with tissue extracts in P.indicus and M. dobsoni as stated above and several other works on crustaceans (Mcleese, 1970, Shelton and Mackie, 1971, Mackie and Shelton, 1972; Mackie, 1973; Carr, 1978; Heinen, 1980; Zimmer

- Faust and Michel, 1980; Carr and Derby, 1986 b) indicate the importance of low molecular weight substances, especially amino acids; on chemoreception in decapods.

Both P.indicus and M.dobsoni are highly sensitive to certain amino acids like L-lysine, L-glycine, L-proline, L-alanine, L-phenylalanine, L-tryptophan, ornithine, L-methonine, L-isoleucine, and taurine and were detected by both species at very low (10^{-6} to 10^{-10}M) concentration whereas amino acids like L-cystine, L-valine, DL-2-amino-n-butyric acid and DL-aspartic acid were detected only at higher concentrations. (10^{-2}M) (Table 23). Unfortunately, it is very difficult to compare the present data, with those reported for other crontaces due to the wide variation in the experimental methodologies followed and due to species diversity. The threshold reported to date for crustaceans (10^{-4} to 10^{-10}M) vary widely and are too high to directly explain the observed behavioural chemosensitivity (Levandowsky and Hodgson, 1965; Ache 1972, Shepheard, 1974; Fuzessery 1978). Nakamura (1987) observed that the threshold of arginine and glycine for P.japonicus 2 x 10^{-10} M lower than the values observed for P.indicus and M.dobsoni. Carr and Gurin (1975) found that in P.pugio, taurine (4 x 10^{-3}M), glycine (5x10^{-3}M) L-glutamic acid (10^{-2}M) and betaine (10^{-2}M) were response inducers. Several workers have reported the lowest threshold of most stimulatory aminoacids for other crustacenas like H.americanus which were between 3.5 x 10^{-6} to 3.5 x 10^{-4} (Derby and Atema, 1982 a) P.argus, 10^{-12}M a (Price and Ache, 1972) and Portunus pelagicus 2x10^{-7} to 2x10^{-5}M (Archdale and Nakamura, 1992). The detection threshold obtained for major amino acids in both species were lying well within this range specified above for other crustaceous.

In most of the cases the detection threshold for P.indicus is lower than that of M.dobsoni, the reasons for which is not well known.

Both P.indicus and M.dobsoni are more sensitive to L-forms of amino acids than DL-forms (Fig.16). For eliciting the same amount of response DL-forms require higher concentration than L-forms. The detection threshold of DL-forms is also several fold higher than that of L-forms (Table 23). Similar observations were also made by several other workers in crustaceans, like Homarus gammarus (Mackie, 1973) and Cancer antennaris (Case 1964) and in balanus, Balanus hamperi (Allison and Dorsett, 1977). The higher activity of L-isomers of amino acids are well known.

The most stimulatory amino acids for P.indicus are L-lysine, L-methionine, L-glycine, L-alanine, and L-proline, and for M.dobsoni they are L-lysine, L-methionine, L-alanine, L-phenylalanine, and L-leucine (Table 25). Betaine and valine produced very weak response in P.indicus and no response in M.dobsoni. The rank order of mean activity at the same level observed in this study differed clearly from that reported in previous studies in other crustaceans. The most extensive survey of amino acid sensitivity by Case (1964) and Shepheard (1974), ranked glumatic acid, taurine and aspartic acid as strong stimulants. Whereas Heinen (1980) and Bauer and Hatt (1980) ranked glumatic acid, glycine-betaine and taurine as the most generally stimulatory single substances for decapods. The rank order reported for the lobster H.americanus are alanine, beta-alanine, glutamic acid, proline and succinic acid (Mc Leese 1970), and for Penaeus japonicus as glycine, taurine and serine Deshimaru and Yone 1978.

Certain amino acids like proline and ornithine having very low detection threshold in both the species failed to produce the corresponding feeding

response in the behavioural evaluation. These amino acids might have some role other than eliciting feeding response in P. indicus and M.dobsoni. Taurine produced higher feeding response in P.indicus but poor response in M.dobsoni. As stated above both species differ in other response to amino acids and so the rank order also. This variation in the response of shrimps to different amino acids may be due to the differential adaptation of their chemoreceptor systems to different amino acids which are rich in their potential food. Several other workers have also reported such a species specific variation in other decapods. (Hindley, 1975; Ameyaw-Akumfi, 1977; Roberston, 1980; Roberston et.al., 1981; Nakamura, 1987; Tierney and Atema, 1988). But such later specific comparison of chemosensory ability are difficult because of experimental techniques, and especially criteria vary greatly and are of the different phylogency. But most of them are of the opinion that each species has a proper receptor for specific amino acids and also have adaptation to different food in their limited foraging environment.

Responses of P.indicus and M.dobsoni increased with the aminoacid concentration (Fig 14 a & b). But the actual increase in response was not linear with that of the increasing stimulant concentration. Fuzessery et.al(1978) also observed a series of roughly parallel lines in response to increasing stimulus concentration in P.argus. But many have reported a bell-shaped dose-response, in which the response increased with concentration upto some level and then decreased at higher concentration in Crangon crangon (Dahm, 1975) P.pugio (Carr, 1978) in lobsters H.americanus (Mc lese, 1970) and P.argus (Carr, 1978) and in crab Carcinus meanas (Shelton and Mackie, 1971).

Some amino acids perform all positive feeding functions in P.indicus and M.dobsoni, but progressively a higher concentration is needed for action as

attractant, arrestants, incitatns and ingestants (Table 26). There are also evidences in other crustaceans that a hierarchy of behaviour can be evoked by different stimulus concentrations (Hindley, 1975; Pearson and Olla, 1977, Pearson, et.al., 1979). In P. merguiensis Hindley (1975) observed that some amino acids at low concentration (10^{-6}M to 10^{-5}M) function as attractant and at higher concentration (10^{-2} M to 10^{-1}M) as incitants. One possible explanation for this is that there is differential access of stimulus molecules to the receptor sites, which may effectively lower both physiological and behavioural thresholds (Pearson and Olla, 1977; Pearson et.al., 1979). Another possible explanation given by Atema (1977) is that, the neural activity in the central nervous system controlling the grasping and ingestion response might be elicited only with higher concentrations of chemical stimuli, than that controlling searching behaviour.

Post larvae, juveniles and sub-adults of P.indicus and M. dobsoni differed significantly in their sensitivity to chemical stimuli (Table 24). Juveniles are more sensitive than sub-adults and post-larvae. Post-larvae detect all stimuli only at a higher concentration, than the other age groups. This higher responsiveness of juveniles may be due to the fact that they are more euryhaline and stronger osmoregulators than other groups. Juveniles of penaeid shrimps are more euryhaline and stronger osmoregulators than adults (Cheng and Liao, 1986; Chien, 1992). Moreover they are in a fast growing phase, with various feeding habit. These two factors may work together and make the chemoreceptor system of juvenile shrimps more sensitive to feeding stimuli.

3.2 SUGARS

P.indicus and M. dobsoni were also sensitive to sugars but the responses were very weak when compared to amino acids (Table 23, 25 Fig 18). Similarly Burney and Sieburth (1977) reported that for most of the aquatic marine crustaceans carbohydrates is a poor feeding signal, because they are present in high back ground noise in the sea water. In P.japonicus sugars, cellobiose and galactose showed to be high stimulant, but was weaker when compared to amino acid (Nakamura, 1987). But in contrast to shrimps, carbohydrates form the major stimulants in crabs (Hartman and Hartman, 1977; Zimmer et.al., 1979; Roberston et.al., 1981; Trott and Roberston, 1984) in cray fish, Procambarus simulens (Ashby and Larimer, 1965) and in antarctic krill (Harner et.al., 1983).

Thresholds for glucose and sucrose ranged between 2×10^{-2} to 9.5×10^{-4}M; indicating 10,000 to 1,00,000 lower sensitivity compared to the average threshold of sensitive aminoacids. In P.japonicus the threshold for various carbohydrates were 2×10^{-9} to 2×10^{-8} M indicating a 10 time lower sensitivity compared to aminoacids (Nakamura, 1987). Whereas in crabs they have higher stimulatory effect and their threshold has been recognised as higher than amino acids by 100 to 1000 times (Hartman and Hartman, 1977). These findings indicated that sugars have only limited role as feeding attractant in shrimps, whereas it forms the major feed attractant and stimulant in crabs.

4. WATER QUALITY PARAMETERS ON CHEMORECEPTION

4.1 EFFECT OF SALINITY ON FEEDING RESPONSE

P.indicus and M. dobsoni elicited typical feeding behaviours at all the salinities tested, but the intensity of feeding response varied slightly with salinity changes (Table 27 to 80). The feeding responses were high at 20%. salinity and least at 5%. and 35%. salinity in P.indicus and at 15%. and 35%. salinity respectively for M. dobsoni Although both species can tolerate wide ranges of salinity, the ideal salinity for the optimal feeding response was between 15%. and 20%. salinity. Cheng and Lia (1986) stated that osmoregulation and balance of body fluid was performed at salinity above 15%. in sub-adults of penaeid shrimp. In P.monodon the optimum salinity for normal growth performance of P.monodon is between 15-30%. (Chen, 1976; 1985). These findings indicated clearly that the chemoreception will also take place effectively at salinity ranges between 15 and 20 ppt in penaeid shrimps.

In both the species the most ideal salinity range for an optimum chemotactic activity and feeding response changes slightly during their life history (Tables 27; 28; 29; & 32). The time lag to respond was minimum and response was maximum at 20%. salinity for post larvae of both species, but for juvenile and sub adults of P.indicus it was 25%. and of M. dobsoni 15%. Such a change in the optimum salinity levels during various stages of P. monodon life history was reported by several workers (Valencia, 1976, Chen, 1976; 1984). They stated that though penaeid shrimp tolerate wide ranges of salinities their growth and other activities are ideal at certain optimum levels, during different stages of growth. This study also indicated that juveniles are chemotactically more sensitive than post-larvae and sub adults;

and also that the chemosensory system of post-larvae are more adapted to lower salinity then other stages.

The feed intake by the test animals varied slightly with the salinity (Table 32). The post-larvae and juveniles of P.indicus showed maximum feed intake at 20%. salinity and the sub-adults at 25%. but in M. dobsoni it was at 20%. for post-larvae, 15% for juveniles and sub adults. Here also the variation in salinity preference by different stages of animals were clear. Deshimaru et.al. (1985) reported that there was little difference in the feed efficiency for P.monodon, between different salinity levels tested, but they observed better growth and survival at 5%. and 20%. salinity. These results indicated that salinity has some limited role on the feeding response and feed intake in penaeid shrimps.

4.2 EFFECT OF pH ON FEEDING RESPONSE

Behavioural responses of the shrimps P.indicus and M. dobsoni to feeding stimuli varies considerably with changes in pH (Fig 21, Table 34, 35 & 36). Feeding behaviour was more intense and frequent at pH 8.0 and minimum at extreme pH levels of 6.0 and 10.0 studied, beyond which no significant response was observed. When the pH was increased or decreased from the optimum level, the perception bahaviour, such as antennular flicking, orientation behaviour and grooming movements decreased significantly. The shrimps which were once exposed to extreme pH even after its transfer to normal pH, continued to show only reduced activity for several days. This reduction in the chemotactic response at extreme pH level may be due to the damage of chemosensory epithelial cells of aesthetasc hairs, thus impairing its chemosensitivity. Literature on the effect of pH on chemoreception in crustaceans is very scarce. Tierney and Atema (1986 a & b) stated that

lowering of pH to the acidic side significantly reduced the feeding response in crayfishes, Procambarus acutus and Orconectes viridis. Similar pH dependent variation in the chemoreception was also reported in the crayfish (Hatt, 1984) and in several fish species (Hara, 1976; Lemly and Smith, 1985).

The time lag to elicit various feeding movements and the time spent in performing various activities reduced significantly with increase or decrease of pH from optimum level (Fig 20 (a to d); Table 33). Tierney and Atema (1986 b) also observed a similar reduction in the time spent in performing different feeding movements in crayfishes, with reduction in the pH. According to Tierney and Atema (1986 a) the reduced feeding movements when exposed to acidic pH probably results specifically from reduced ability to detect and/or respond to these stimuli, rather than by being induced by the reduction in pH. They supported Hara's and Bauer et.al.'s model for fishes of changes in amino acid structure and receptor binding at lower pH levels.

A change in the pH of the rearing medium from optimum level significantly reduced the amount of feed actually consumed by the shrimps (Table 37). The maximum feed consumption was observed at pH 8.0 and minimum at pH 6.0 and 10.0 for both species. At pH 6.0 and 10.0 the feed consumption reduced by 50% than that at pH 8.0 and above 10.0 and below 6.0 no intake was observed. Similar pH dependent variation in the feed intake was reported in P.monodon by Clifford (1992). At pH between 9.0 and 9.5 the feed consumption reduced by 25%; between 9.5 to 10.0 by 50% and above 10.0 no feed intake was reported. Law (1988) also stated that the ideal pH for optimum growth performance of P.monodon on between 7.5 and 8.5.

These studies indicated that exposure of shrimps to extreme pH levels at acidic and alkaline range has pronounced influence on the feeding response. Exposure to these pH levels interfere specifically and quantitatively with chemoreception process in P.indicus and M. dobsoni.

This study also showed that the feed coated with a stimulant was more effective in eliciting ingestion than an uncoated feed (Table 37). Coating feed with an attractant or stimulant significantly reduced the time required to attract the shrimp to the feed and thus initiate ingestion in a short time (Fig 20). It also increased the amount of feed consumed per unit biomass of the shrimps, compared to uncoated diet.

5. EFFECT OF STARVATION ON FEEDING RESPONSE

The feeding response and the time lag to elicit different feeding behaviour in shrimps varied significantly with the degree of starvation. With increase in the degree of starvation the feeding response increased initially upto a certain level of starvation, beyond which it decreased (Fig 22). Similarly, the time lag to elicit various feeding behaviours, decreased initially up to 8 - 10 days of starvation, but thereafter the time lag again increased in both species (Fig. 23, 24). A similar increase in the behavioural responses to chemical feeding stimulants have been reported to accompany food deprivation in H.gammarus (Mackie and Shelton, 1972), P.pugio (Carr et.al., 1984) P.vannamei (Costero and Meyers, 1993) and Callinectes sapidus (Pearson and Olla, 1977).

As the rate of starvation increases the shrimps showed increased grooming and probing movements even in the absence of any chemical feeding stimuli. At this stage even a weak stimuli produced very intense feeding response, in a

very short time lag. Symonds (1964) reported such an increase in the frequency of behaviours like probing, grooming and distance travelled in Hemigrapsus origonesis, after starvation. Such arise in the probing and grooming movements increases the chance of finding food. But the exact reason for the fast response, of the starved animals, i.e. reduction in the time lag to elicit feeding response is not clearly known. It may be due to the fact that a starved animal may be on the look out of a food and thus even a weak stimulus may evoke quick response, due to decrease in the behavioural threshold of shrimps towards chemical stimuli. Such a drop in the threshold for eliciting feeding response due to progressive starvation was reported in H.gammarus (Mackie and Shelton, 1972) and in C.sapidus (Pearson and Olla, 1979).

But as the degree of starvation increased beyond a limit, the intensity of grooming and probing movements decreased slightly with starvation and animals were found to occupy some still position at some corner of the tank; with only occasional weak grooming and prbing movements. At this stage the animals also responded only very weakly to the feeding chemical stimuli. This continuous drop in the feeding response towards the advanced stage of starvation was due to the general weakness of animals as a results of starvation. In the case of young ones of both P.indicus and M. dobsoni, especially post-larvae, most of them died after one week starvation and only few survived, for further evaluation.

The feed intake of the starved animals also followed the same pattern as that for feeding behaviour. The feed intake increased with the degree of starvation initially up to one week; beyond which the same declined, gradually with degree of starvation. The reasons for such a reduction in feed intake is

not well understood. But, it may be due to the general weakness cased by starvation, since the starved animals showed only weak and delayed response to chemical feeding stimuli.

6. FEEDING CHEMORECEPTOR SITES

6.1 LOCATION OF THE CHEMORECEPTOR SITES

The major sites inolned in the reception and detection of chemical feeding stimuli in P.indicus and M. dobsoni are antennule, mouth appendages including maxilliped - III and the chelae of first three pairs of walking legs (Tables 39 & 40). Antennae have no role in chemoreception and vision was not involved in the detection and location of food, in both species. Hindley (1975) postulated that P.merguiensis detects food, discriminates it from non-food particles by chemosensory mechanisms, and that chemoreceptors are distributed over the body surface and concentrated at the anterior end. Hindley (1975) and Derby (1982) stated that antennae is also involved in chemoreception in crustaceans, in contrast to the present findings, that antennae have no role in the same. But many workers through morphological and behavioral studies in other crustacean species found that only antennule, mouth parts including maxillipeds and walking legs are involved in chemoreception. (Spiegel, 1927; Case and Gwilliam, 1961; Levandowsky and Hodgson, 1965; Ache and Case, 1969; Hazlett, 1971 a; b; Field, 1974; Trott and Roberston, 1984). They found that crustaceans showed heavy reliance on their antennules for locating food from a distance.

Absence of any one of these appendages showed to reduce the effectiveness of chemoreception in both species to some extent only, because many sites are

involved in the same function, except the absence of antennules alone which was found to impair the effectiveness of orientation to a greater extent, (Table 41, 42 and 43; Fig 25 (a-c)). Hence the elicitation of other behaviours were also delayed and weakened. Similarly, Ameyaw-Akumfi (1977), stated that, absence of any of these appendages did not have any effect on the chemosensory ability of the crayfish, P.clarkii to perceive food.

In the absence of antennules, walking legs and mouth appendages, both P.indicus and M. dobsoni were found to elicit some weak orientation behaviour in response to strong chemical feeding stimuli, indicating the presence of some other weak distant chemoreceptor sites in shrimps. There exists a possibility like some chemoreceptive sites along the path of respiratory sites. Such a chance was also suggested by Ameyaw-Akumfi (1977) in freshwater crayfish P.clarkii.

6.2 STRUCTURE AND FUNCTION OF CHEMORECEPTOR SITES

Many workers have given detailed morphological descriptions and functions of major chemoreceptors in many crustaceans. A similar detailed structural and functional investigation have been carried out for the chemoreceptor sites of P.incidus.

6.2.1 **Vision :** In the shrimps, P.incidus and M.dobsoni, vision does not seem to influence distinctly the chemical sensitivity. Animals without vision do not exhibit any change neither in the feeding behaviour nor in the latency to elicit the same (Fig 25). But several workers have reported that eye stalk ablation was found to adversely affect the chemical sensitivity of the spiny lobster, P.argus (Maynard and Dingle, 1963; Maynard and Yager, 1968; Maynard and Sallete, 1970); Mollar (1978), also found that the post-larvae of

Macrobrachium rosenbergii utilises visual sense for location and capture of food, along with the chemical and rheotactic senses. The hermit crab Clibanarius vittatus also used visual stimuli, along with chemical stimuli (Hazlett, 1968) for locating the food. He further stated that once elicited, the feeding behaviour can be guided by visual and/or chemical stimuli. Maynard and Dingle (1963) stated that there is an integrating centre in the proximal ganglion of the eyestalk of spiny lobstor, involved in the chemoreception.

But the present study and several workers have strongly suggested that vision has no distinct role in chemoreception and feeding responses in the shrimps P.japonicus (Nakamura, 1987) and P.merguiensis (Hindley, 1975) and in the shore crabs Hemigrapsus oregonensis (Hiatt, 1948; Symons 1964) and Carcinus maenas (Bethe, 1897). Several workers also stated that visual and tactile stimuli often are without effect while odours released from prey cause marked food searching response (Derby and Atema 1981; Zimmer-Faust and Case, 1982 a). Hindley (1975) is of the opinion that since food of penaeid shrimp in general are mainly organic debris and bacterial colonies it is probable that food would have any visually recognisable features.

But it was possible that vision may lead shrimps towards objects, that could be food, in the vicinity, but it does not appear to have any important sensory modality for the detection of food, as has been observed by Cowels (1908) in the crab Ocypoda arenaria.

6.2.2 **Antennae :** Results of the present study indicated that antennae have no significant role in chemoreception and feeding response in the shrimps P.indicus and M. dobsoni. Contrary to the present observation several workers have considered antennae along with antennule as an important site of

1978; Devine, 1981; Devine and Atema, 1982) and P.argus (Laverack, 1964; Ache, 1976; Reeder and Ache, 1980). They observed that unilateral ablation of laternal antennular filament impaired the orientation ability of lobsters, whereas medial filament has effect on their orientation ability. In the freshwater crayfish, Cambarus bartoni sciotensis (Hodgson, 1958) and in the lobstor, H.americanus (Devine and Atema, 1979) medial filaments were observed to be involved in chemoreception. Devine and Atema (1979) reported that ablation of lateral flagellum of antennule did not impair the lobster's ability to detect the presence of stimulus nor altered the alert time. But the ablation of medial flagellum significantly altered the alert time.

In the present study with P.indicus and M.dobsoni, ablation of any of the antennular flagellum only slightly reduced the orientation ability and marginally increased the time lag to elicit response behaviour. But ablation of both the flagellae together; completely impaired the orientation ability; indicating the role of both filaments in chemoreception, especially in perception and orientation behaviour (Table 41 & 42). Similar chemosensory role of both the antennular filaments in other crustaceans have been reported by many workers (Hodgson, 1958; Mackie, 1973, McLeese, 1973; Fuzessery, 1975, 1978, Hindley, 1975; Fuzessery and Childress, 1975, Schmidt and Ache, 1979; Thomas and Ache, 1980). But even after the ablation of both antennules of shrimps some weak perception and orientation behaviour towards chemical feeding stimuli, indicated the existence of some other chemoreceptor sites having distant chemoreception property. Since loss of appendages is common in crustaceans, this partial overlapping of organ function may serve the animal well in nature. Hazlett (1971 b) stated that dactyl sensitivity increases considerably after ablation of antennules in hermit crabs. The observed orientation response in P.indicus and M. dobsoni after antennular ablation may

be due to the increased sensitivity of other chemoreceptor sites or due to the presence of other sites involved in chemoreception.

The behavioural observation of P.indicus and M.dobsoni have shown that antennules play a significant role in the perception of chemical stimuli and orientation of animals to stimulus source and their function as a distance chemoreceptor is indicated by the sharp increase in Et50 value when ablated and a small Et 50 value when exposed individually (Table, 43). Antennules of marine decapods have been shown to be the main distance chemosensory organ involved in perception and orientation towards chemical feeding stimuli through morphological, behavioural and electrophysiological studies (Bell, 1906; Copeland 1923, Brock, 1926; Hodgson, 1958; Maynard and Dingle, 1963; Laverack, 1964; 1968; Hazlett, 1968, 1971a, Ache, 1972; 1975; Mcleese, 1973 a, 1973 c, Thompson and Ache, 1980; Derby and Atema 1982 b). These workers have reported that chemoreceptors of antennules are highly sensitive and will detect even very low concentrations of stimuli thus supporting its distance chemoreceptor property.

Two types of setae have been observed along the antennular flagella of P.indicus (Plate 1 and 2). Scanning electron microscopic observations revealed thin walled peg settae with a pore at the tip (Plate 2). The other type is sturdy and narrow towards the distal end with a pore at the tip (Plate 1). The thin walled setae are said to be highly sensitive and is involved in distance chemoreception. The other setae type is also found to be chemoreceptive from its structure but its exact role is not well understood. Similar thin walled aesthetase heirs with sensory pore have been reported on the antennular flagellum of decapods by several workers (Laverack, 1964; Laverack and Adrill, 1965; Ghiradella et.al., 1968; Lindstedt, 1971;

Shepheard, 1974; Fuzessery, 1975; Fuzessery and Childress, 1978; Reeder and Ache, 1980; Devine, 1981; Derby, 1982; Devine and Atema, 1982). But in lobsters three distinct type of hairs have been reported by several workers (Laverack 1964; Derby 1982). Of these the thin walled aesthetasc hairs have been protected by companion and guard hairs. The setae types found on the basal segments (Plate 3, 4 and 5) may have some supplementary role in chemoreception, since it was observed that once excited with a chemical stimuli, these setae also splays out along with aesthetasc hairs.

The aesthetasc hairs function in feeding behaviour by constantly providing information about the chemical cues in the water in which the animal lives. Animals flick the antennules occasionally when they are resting and continuously when exposed to chemical stimuli. Flicking cause circulation of water around the sensory hairs and disturb the existing concentration gradient, which would enable the animals to constantly restimulate with a high or low intense stimuli. The role of flicking in chemoreception have been variously explained by several workers; it increases the passage of water over the chemoreceptors (Snow, 1973a); prolongs the period over which animals is sensitive to the stimuli (Price and Ache, 1977), enhance reception (Fuzessery & Childress, 1975; Schmidt and Ache, 1979)

6.2.4 **Walking Legs:** The first three pairs of walking legs in P.indicus an M.dobsoni are chemosensory involved in the food picking, discriminating food particles from non-food items and transfering it to the mouth. (Table 39 & 40). Similar positive chemotactic property of walking legs of decapods have been reported by several workers (Spiegel, 1927, Luther, 1930; Butler, 1954; Levandowsky and Hodgson, 1965; Ache and Case, 1969; Hazlett, 1971 a, b; Ameyaw-Akumfi 1977; Pearson et.al., 1979). But, Derby (1982) reported that

all the five pairs of walking legs in H.americanus is chemosensitive and according to Hodgson (1958) in the crayfish, C.bartoni sciotensis only the first two pairs of walking legs are involved in chemoreception.

In P.indicus and M.dobsoni with coated dactyls and propodus, the food picking behaviour was not observed (Table 41 & 42), indicating that leg chemoreceptors on the dactylus and propodus are essential for releasing grasping response. Similar impairment of grasping response was also observed in H.americanus (Derby and Atema, 1982 b). Several other workers also stated that chemoreceptors of walking legs are concentrated on the propodus and dactylus, which is responsible for releasing grasping response and that they represent the sense of taste in crsustaceans (Bell, 1906; Luther, 1930; Case and Gwilliam 1961, Case 1964, Shelton and Laverack, 1968; 1970; Ai and Takei, 1973 b; Pearson et.al., 1979; Derby and Atema, 1982 a; Devine and Atema, 1982 b). They stated that the shift from searching to grasping is dependent on stimulus on the dactylus and propodus of walking legs. The present observations and previous results indicated that chemoreceptors on the dactylus and propodus of the walking legs have higher threshold and is responsible for contact chemoreception. Several workers also made similar observation that crustacean chemoreceptors have higher threshold than antennular receptors (Case and Gwilliam, 1961, Shelton and Laverack, 1970; Ache, 1972; Shepheard, 1974; Fuzessery and Childress, 1975; Fuzerrsy et.al., 1978). But Derby and Atema (1982 a) in contrast to these findings reported that leg chemoreceptors of H.americanus have lower threshold similar to the antennular receptors. Chemoreceptors on the dactyls and chelipeds would constantly provide information about the substratum over which the animals range. Shrimps frequently search the substratum with their chelipeds as they move or groom in response to feeding stimuli. Such a feeding behaviour was

also reported in ghost crab, when they are stimulated with potential feeding stimuli (Trott and Roberston, 1984). The crabs as they move tap the substratum with their major chiliped.

Electron microscopic observations revealed three types of complex cuticular structures on the propus and dactyl (plate 6); some of which are chemosensory, some are mechanoreceptors and others have cleaning function. The position of the simple setae over the surface of the chelae, and its structure suggested that they are chemosensory involved in contact chemoreception and is responsible for discriminating edible from non-edible materials (plate 7,8). Specific chemosensory sensilla on dactylus and propodus have been identified in crayfish (Hatt and Bauer, 1980) and in the lobsters Panulirus interruptus (Drach and Jacques, 1977) H.gammarus (Shelton and Laverack, 1968, 1970) and H.americanus (Bauer, 1975; and in P.clarkii (Mittenthal, 1981, Derby, 1982). Several workers also ascribed similar chemosensory function in the tufts of simple setae over the dactyls of walking legs in other crustaceans (Case and Gwilliam, 1961; Case 1964; Ai and Takei, 1973 b., Hindley, 1975, Lindsey, 1976). Whereas these tufts of setae are mechanosensitive in crayfishes (Hatt and Bauer, 1980; Riemay 1980).

The branched setae found on the carpus have all the characteristics of a chemosensory setae, involved in distance chemoreception (Plate 15). These setae types are responsible for perception and orientation in the absence of antennular receptors in shrimps. It might have been about the setae that Derby and Atema (1982 a) stated that leg chemoreceptors have lower threshold similar to antennular receptors. This has also probability that the sensitivity of this setae may increase after antennular chemoreceptor inactivation or antennular ablation as has been stated by Hazlett (1971 b).

The other setae types found on the walking legs of P.indicus probably have mechanoreceptive and cleaning functions. The pegs and ridges on the jaws of chelae (Plate 9) are mechamoreceptive devices with particle size discirminating function. In crayfish (Hatt and Bauer, 1980) they have chemosensory role, whereas in the lobsters (Shelton and Mackie, 1970; Derby, 1982) have both chemo and mechanosensory functions. Hindley and Alexander (1978) stated that in P.merguiensis they are of unknown function. But Laverack (1964) stated that in P.argus they have only mechanosensory role, responsive to water current and touch. The denticulate pads on the inner side of the chelae are the devices for firmly gripping objects rather than testing objects (plate 10).

The serrated setae seen on the articulation of the propus and carpus of the first walking leg are involved in the cleaning of body surface and other appendages (Plate 11, 12, 13). This variation in the size and structure of the setae enable the cleaning of different parts of the appendages efficiently. The serrate setae are known to have cleaning and chemosensory function in Pandalus danae (Bauer, 1975; 1981) and in H.americanus (Derby, 1982) but Shelton and Laverack (1970) stated that in H.americanus they have only chemosensory function.

6.2.5 **Mouth Parts:** The behavioural observations in P.indicus and M.dobsoni indicated that mouth appendages have both distance and contact chemoreceptor property (Table 39-43, Fig. 25). Analysis of the role of crustacean mouth appendages in chemoreception and feeding response have been fewer largely as the result of conducting behavioural observations on living animals regarding each chemoreceptor site. Most of the previous interpretations have relied on

interpreting behaviours from the morphology and electrophysiological observations.

In contrast to the present findings in P.indicus and M. dobsoni, Hodgson (1958) reported that mouth appendage of crayfish C.bartonis sciotensis have no chemosensory role. But positive chemosensory role of mouth parts have been reported by many in crayfish (Bell, 1906), brachiuran crabs (Luther, 1930), Carcinus maenas (Case and Gwilliam, 1961) H.americanus (Derby, 1982; Derby and Atema, 1982 b) H.gammarus (Shelton and Laverack, 1968; P.argus (Fuzessery and Childress, 1975) and in P.merguiensis (Hindley, 1975), Derby (1982) reported that all the six pairs of mouth appendages are chemosensory in H.americanus.

Mandible : The role of mandible in feeding and the morphology of the setae types on the mandibular palp suggested that they are strong chemoreceptors. The masticatory role of mandible in feeding suggested that they may possess contact chemoreceptors involved in taste reception. But the morphology of the setae on th mandibular palp (plate 17; 18) lead to the conclusion that there might have been some distance chemoreception property also. The contact chemoreceptor properly of mandibles have been supported by the findings of Herrnkind (1968) to the zoeae of Uca pugilator and Moller (1978) in the larvae of Macrobrachium rosenbergii, that they tested the particles captured through chance encounter through biting, suggesting that taste receptors are concentrated in the mandibular region.

Maxilla I and II : The morphology of the setae on the protopod of the maxillule (Plate 19) and their close association with mandible suggests that they are contact chemorceptors involved in taste reception in shrimps.

Similar information on the role of maxillae in chemoreception from other crustaceans are not available.

Maxilliped : Electron microscopic study indicated that in P.indicus maxilliped 1 and 3 function mainly as contact chemoreceptor and maxilliped - 2 as distance chemoreceptor.

Maxilliped 1 : Tubular spine like setae with pore at its distant end, along the propus suggested that they function as contact chemoreceptor responsible for close range detection of chemical stimuli (Plate 21).

Maxilliped 2 : Long, slender, thin walled setae along the groove of propus (Plate 23) due to its morphology suggested that they are typical distance chemoreceptors involved in perception and orientation.

Maxilliped 3 : Behavioural evidence indicated that maxilliped - 3 is chemosensory in both P.indicus and M. dobsoni (Table 39, 40). Electron microscopic observations, revealed short, stumpy, spine like setae on the propus (Plate 26) probably responsible for contact chemoreception. Food picking activity by maxilliped - 3 in the absence of functional pereopods, and the initiation of ingestion when the maxilliped makes contact with the food observed in the present study suggested that they bear contact chemoreceptors.

Literature on the role of maxilliped in chemoreception and feeding behaviour is very sparse and widely distributed. The role of these appendages on feeding in shrimps have been described by many workers (Hindley and Alexander, 1978; Alexander et.al., 1980). Luther (1930) considers maxilliped as the inner contact chemoreceptor involved in reception in brachurans. The findings of Ameyaw-Akumfi(1977) in Procambarus clarki and of Derby and Atema (1982 b) in H.americanus indicate the presence of chemoreceptor sites in the maxillipeds.

Propodus and dactylus also bear different types of cleaning setae as that of the pereopods (plate 24, 25). They have no role in chemoreception. The setae present on the exopodites (Plate 27) also have no chemosensory function, instead they may aid filter feeding, manipulation of large food particles, and cleaning of other appendages.

Pleopods, rostrum, telson, uropod and general body surface do nothave any chemosensory role either morphologically nor behaviorally in P.indicus and M.dobsoni. But a very weaker feeding response observed in the present study when all the chemoreceptor sites were blocked indicate the presence of some other insignificant chemoreceptor site like ciliary lining of gills; as has been reported by Zimmer et.al.(1979) in the crabs, Pugettia producta.

6.3 STRUCTURE OF THE SENSILLA

Different types of setae are known in crustaceans, especially on the antennules, mouth parts and walking legs. In the present and most of the previous study sensory function of setae are inferred from the behaviour of the animals and surface features on the setae themselves, such as presence of pores, tanned cuticle or reticulated base.

Several workers have reported that in decapod aesthetasc, the dentrite branches extensively and then gradually loses its integrity. (Ghiradella et.al., 1968; Snow, 1973 b). Several works on aesthetasc revealed an apical pore, which allows stimulus entry (Laverack and Adrill, 1965; 1966; Dahl, 1973; Neilson and Stromberg, 1973; Lindsey, 1973; Shelton, 1974, Ball and Cowan, 1977). But several workers proposed that the presence of pores in crustacean setae is associated with moulting or abrasion (Anderson, 1975, Guse, 1980; Hamilton, 1980). But the present study showed that pores are

present on the setae irrespective of moulting stage; and this itself suggested that it is not due to abrasion.

Electron microscopic observation of chemoreceptors revealed basically two morphologically distinct type of setae. One type is thin walled, permeable along its length either branched (Plate - 15, 17 & 23) or unbranched (Plate 2) with a "sensory" pore at the tip. The other type is thick walled permeable at the tip through a sensory pore (Plate 1, 7, 8, 19, 21, 26). But depending upon their location their size and structure varies considerably. Slifer (1970) also concluded that Aquatic crustaecans two basic types of chemosensory setae. He further stated that the thin walled sensilla are innervated by large number of receptor neurons with branched dendrites and the other types are innervated by fewer number of receptor neurons, with unbranched dendrites.

7. INGESTIVE PROPERTY OF NATURAL AND CHEMICAL STIMULI

7.1 AGAR MATRIX PALATABILITY BIOASSAY

A feeding bioassay which uses agar discs was developed for evaluating chemosensory stimuli influencing ingestive behaviour in P.indicus and M.dobsoni. The palatability assay required only small amounts of stimuli and is suitable for rapid and inexpensive screening of a wide variety of compounds. The added advantage of the system is that only quite small amounts of stimuli can be used to test large number of individuals. Agar gel hardness prevents the shrimp from placing their mouth parts directly on the stimulus and this is one of the important aspect of the bioassay design. Unlike feed tests which incorporate stimuli into feed pellets, this assay is independent

of confounding factors such as pellet size, texture, or hardness, and the results are not influenced by other compounds extent in the pellets.

This tests allow direct comparison of the relative efficacy of the paired stimuli and gives good indication of relative rank and general preference trends. For the tissue extracts tested equal weights with P.indicus, the palatability ranking was Squid>Crab>Clam>Oyster>Shrimp extract, and for M.dobsoni it was Squid>Shrimp>Clam>Crab>Oyster extracts (Table 44,45). The palatability ranking of the extracts differed clearly from response ranking based on the feeding response, in both the species. This difference may be due to the fact that the factors eliciting feeding behaviour will be different from those eliciting ingestion activity in shrimps. Nakamura (1987) reported that specific amino acids elevate only orientation and searching behaviour in P.japonicus, but for ingestion response, some proteins such as casein and gelatin as well as saccharides, glucose and starch were more stimulative than amino acids. So the present variation observed between the feeding response and ingestion rate elicited by the same extract may be due to the difference in the relative composition of various components in the extracts.

As has been reported earlier, the incorporation of squid ink in the gel decreased its palatability in both the species, confirming the presence of feeding repellent in the squid ink (Table 44 and 45).

Tissue components significantly differ in their ability to elicit feeding activity and feed consumption in both species (Table, 46 and Fig. 26,27). These present study indicated that free amino acids form the major flavour component of tissue extracts eliciting ingestion activity in both species. The second major component of tissue extract is the nucleotide, followed by the soluble proteins. The ingestion activity elicited by the extract components

followed the same pattern in that observed in the behavioural trial. But the difference observed between the extracts sources is due to the variation in the relative composition of each fractions and also due to the variation in the composition of individual components in each fractions.

At the same concentration neutral amino acids and basic amino acids produced maximum ingestion activity and agar consumption in both, P.indicus and M.dobsoni (Table 47a) Aromatic and acidic amino acid product almost similar level of activity. The reasons far the agar activity of neutral and basic amino acids are not well understood. It may be due to variation in stimulus and receptor interactions.

Among the nucleotides; IMP and inosine produced the maximum agar ingestion and relation activity in P.indicus and IMP and AMP in M.dobsoni (Table 476). All other nucleotides and related compounds have only limited role in eliciting ingestion activity in shrimps. Literature on the role of nucleotides and related compounds on feeding stimulation is sparse and widely distributed. Zimmer - Faust et.al., (1984) stated that nucleotides are not a feeding stimulant for spiny lobsters. But other workers have stated that nucleotides, betaine and other related compounds are essential for the total activity of tissue extract. The present study indicated that nucleotide play an important role in eliciting ingestion in shrimps.

Betaine and sugar are also found to have limited role as an ingestant in P.indicus and M.dobsoni (Table 476). Both of these components also produced very poor response in behavioural evaluation indicating that they have no role in the feeding activity of this shrimps, when tested alone. But several workers have reported that betaine acted is a stimulant in various aspects of

feeding behaviour in diverse crustaceans when tested alone (Hodgson, 1958, Laverack, 1963, Hashimoto, 1967, Carr 1978, Harpaz et.al., 1987 a; b Harpaz, 1988). Betaine HCl was found to be one of the most potent chemo-attractant for M. rosenbergii in behavioural observations (Harpaz, et.al., 1987 a; b) as well as in electro physiological studies (Derby and Harpaze, 1988) and in one of the major components in extracts of fish, crab and shrimp which are readily fed upon by M.rosenbergii likewise Hodgson (1988) and Laverack (1963) reported that betaine is a feeding incitant in crayfish, crabs and lobsters.

Amino acids like methionine, lysine, proline, arginine and aspartic acid in P.indicus and aspartic acid, methionine, leucine, tryptophan and isoleucine in M.dobsoni produced maximum agar consumption and relative activity (Table 47c). These results on comparing with that of the behavioural assay (Table 25) showed that some of the amino acids which induced very high feeding response produced only poor ingestion activity in both species. Similarly amino acids which produced poor responses in behavioural bioassay induced better ingestion activity. This indicated that the stimulus needed for eliciitng feeding behaviour and final ingestion may not be the same. It further indicates the specificity of distance chemoreceptor responsible for perception and eliciting orientation and food seeking behaviour; and the contact chemoreceptor responsible for inducing food picking and ingestion to amino acids are different.

It is very difficult to compare the present data with that of the previous data in crustaceans, as several workers have reported different rank orders for the same species and also for different species. Arginine is one of the most potent amino acid inducing ingestion in P.indicus, but have only a less significant role in inducing feeding response in P.japonicus. Similarly

glycine elicited feeding behaviour in both P.indicus and M.dobsoni, but had only poor ingestant activity. but glycine was considered as the most potent stimulant for P.japonicus (Nakamura, 1987) and P.monodon (Murai, et.al., 1981). In lobsters, Levandowsky and Hodgson (1958) found glycine as less stimulative.

Several workers reported the prime role of amino acids like glutamic acid (Laverack, 1963, 1968, Takei and Ai, 1971, Hashimoto, 1967, Mackie, 1977) L-serine, L-alanine, and L-aspartic acid (Case, 1964, Crisp, 1967, Mcleese, 1970, Shepheard, 1974, Derby and Atema, 1978, Johnson and Ache, 1978). As in the case of arginine, glutamic acid induced only ingestion activity in M.dobsoni, but have no significant role in the ingestion activity when compared to the most potent amino acids selected. Aspartic acid had ingestion activity in both species and alanine acted as feeding behaviour inducer in M.dobsoni and ingestant in P.indicus. But serine had no role in both species either as behaviour inducer or as an ingestant.

Most of the workers reported taurine as the major extract component of prey organisms inducing feeding activities in decapod crustaceans (Case, 1964; Ache, 1972; Mackie, 1973; Shepheard, 1974; Carr and Gurin, 1975; Carr, 1976; 1978; Carr et.al., 1978; Johnson and Ache, 1978; Trott and Robertson, 1984) Taurine was also found as a potent feed ingestant for P.indicus and M.dobsoni in the present study but had a very poor role in eliciting the initial feeding behaviours. Whereas Robbins (1959) reported that taurine is less effective as a feeding stimulant in decapods. Isoleucine induced ingestion activity in M.dobsoni. But had no role as a feeding behaviour inducer in both species as in the case of taurine. Isoleucine as reported as a potent stimulant for Pachygrapsus (Kay, 1971) and Penaeus paulensis(Dos Santos Filho, 1983).

Aspartic acid was observed as the most potent chemostimulant for M.dobsoni. But Deshimaru and Yone (1978) reported aspartic acid as a poor enhancer of palatability in P.japonicus.

Some amino acids functioned as both feeding behaviour inducer and ingestant in both species. Methionine lysine, proline and alanine in P.indicus and Methionine, leucine and trytophan in M.dobsoni. Literature on the similar activity of these aminoacids are scarce. These results indicated that, aminoacids having such dual function will well serve both the function of a potential feeding attractant and stimulant, instead of going for a number of compounds having single activity.

8. EFFECTS OF ATTRACTANTS AND STIMULANTS ON FEED INTAKE

A series of diet choice feeding trials were conducted both in the laboratory and in the field to ascertain the findings of behavioural and palatability bioassay with agar disc. The data on feed intake and assimilation alone will serve as a guide to whether the attractants also act as a feeding stimulants. Hartati and Briggs (1993) also used feed intake as one of the criterion to assess the feeding stimulatnt property of substances for Penaeus monodon.

8.1 LABORATORY TRIAL

Feeding trials using casein diets incorporated with selected amino acids reproduce more or less the same results as obtained in the palatability bioassay using agar disc as described earlier (Table 48). In this study also

both species showed least preference to ornithine and betaine-Hcl, with small Daily Feed Intake (DFI) and relative activity.

Among the nucleotides IMP produced maximum feed intake in both P.indicus and M.dobsoni (Table 49). But a slight variation in the order of preference compared to palatibility bioassay was observed for P.indicus. This change in the activity may be due to the interaction between the nucleotides under test and the constituent of feed; such compounds usually are not present in agar, which is considered as an inert gel. Such an interaction between betaine and some dietary components have been reported by Carr (1978) in P.japonicus.

Synthetic extracts based on the free amino acid and nucleotide composition of tissue extracts produced better feed intake and relative activity (Table 50). The rank order based on the feed intake and relative activity showed a clear deviation from the order of preference observed during the behavioural bioassay with natural extract for both species. This change in activity may be due to several reasons, such as (i) the components of extracts which produced behavioural response may differ from those producing feed intake in shrimp or (ii) the interaction between different components in the synthetic extracts will differ from that of natural extracts due to the absence of certain components in synthetic extracts compared to the natural extract.

The synthetic extract of squid failed to produce same level of activity as that of its natural extract, when supplied at the same level. This may be due to the reason that protein and lipid fraction which has some role in feeding response were not supplemented in the synthetic mixture. Similarly, many workers on crustacean chemoreception stated that artificial mixtures

based on the analysis of natural materials have been less stimulatory to decapods (Mc Leese, 1970; Mackie, 1973; Ache et al., 1978; Carr, 1978; Johnson and Ache 1978; Carr et.al., 1984). Mackie (1973) also stated that in H.gammarus some or all of the substance present in the squid extract contributed to its total activity. Shelton and Mackie (1971) also stated that one or two groups of component of natural extracts were found to be as effective as the whole extract on shore crabs. The present study also clearly indicates that individual components do not equal the stimulatory ability of the whole extract, but they show some additive effect when supplemented together. Such additive effects are also reported for crab C.maenas (Shelton and Mackie, 1971), lobsters, H.gammarus (Mackie, 1973); H.americanus (Mcleese, 1973), P.argus (Johnson and Adums , 1978) in response to extracts of potential food organisms.

The addition of betaine to the synthetic mixture increased its activity (Table 50). Betaine which is a poor attractant and feeding inducer when supplemented alone, has synergestic effect with other amino acids and amino acid mixtures. Such potentiation of betaine was also observed in P.pugio when combined with aminoacid mixtures by Carr (1978). So it may be worthwhile to add betaine alone to feeds, since it might be synergistic with dietary amino acids. However Deshimaru and Yone (1978) observed no increase in ingestion rate when betaine was added to the diets of P.japonicus.

As discussed in the palatability bioassay using agar disc the different groups of amino acid produced the same pattern of ingestion and relative activity, with neutral and basic amino acids producing better feed intake in both species (Table 51).

When amino acid mixtures are used as the stimulant, they produced higher activity in both species than their individual component when supplemented alone (Table 48 and 52). This enhanced activity of amino acid mixtures are due to the synergistic or additions effects between the amino acid components in the mixture. Such potentiation in the activity of amino acid mixtures have been observed in H.americanus McLeese, 1970), H.gammarus (Mackie and Shelton, 1972; Mackie, 1973), P.interuptus (Zimmer - Faust et.al., 1984). P.argus (Johnson and Ache, 1972), P.pugio (Car, 1978), C.maenas (Shelton and Mackie, 1971), and in Uca pugilator (Roberston, et.al., 1981) Synergism is generally recognised to arise atleast in part from the simultaneous stimulation of different chemoreceptor sites each varying in chemical specificities, as has been suggested by (Shelton and Mackie, 1971; Mackie and Shelton, 1972). Synergistic interaction may also occur between certain components extant in the feeds and the components of stimulant mixture.

A considerable reduction in the time required to elicit feeding behaviour and attract shrimps to the feed and to initiate in ingestion has been observed in all the trials when the diets were flavoured with potent chemoattractants. In P.vannamei; Costero and Meyers (1993) observed a 50% reduction in the time required to elicit feeding response, when diets flavoured with chemoattractants were provided than unflavoured diets.

8.2 **FIELD TRIAL**

Multiple choice feeding trials using a commercially formulated diet incorporated with single and various aminoacid contributions indicated that the attractants and stimulants improved feed intake in P.indicus under field conditions (Table 54). The foraging ratio and selectivity index used in this

study to screen amino acids and their mixtures served as an effective tool to screen all compounds having below average activity, though they increased the feed intake compared to control diet.

As has been discussed earlier for the feeding trials with casein based diet, methionine and lysine produced better feed intake under field conditions. When incorporated with glycine and betaine it also produced very high feed intake after lysine, both of which produced comparatively lower feeding performance during palatability bioassays with agar disc and casein diet. This improved feed intake for betaine and glycine incorporated compounded diets may be due to some synergistic interactions of these amino acids with other compounds present in the diet, as suggested by Carr (1978) for betaine, who reported synergistic effect of betaine with other dietary amino acids extant in the feed far P.pugio. Feeding stimulant property of glycine was also discussed by several workers in P.monodon (Murai et.al., 1987) and in P.japonicus (Deshimaru and Yone, 1978, Nakamura, 1987). But argenine (Nakamura, 1987) and taurine (Case, 1964; Mackie, 1978; Carr and Gurin, 1975, Carr 1978; Deshimaru and Yone, 1978; Carr et.al., 1978; Trott and Robertson, 1984; hartati and Briggs, 1993) were reported as most potent feeding stimulants in many crustaceans, produced only comparatively poor feed intake with around 50% relative activity compared to squid extract.

As discussed earlier several amino acid combinations have shown synergistic effect and produced very high feed intake compared to their individual constituents. But several other combinations produced only poor feed intake than their component amino acids indicating antagonists activity (Table 54). Similar antagonism has been reported by several workers in other crustaceans like H.americanus (Mcleese, 1970) with alanie and arginine, P.argus (Johnson and Ache, 1978; Ache et.al., 1986) with proline, arginine,

alanine and taruine and P.monodon (Hartati and Briggs, 1993) with betaine and glycine. The antagonism may be due to the competition between amino acids for a common receptor site thus decreasing their activity each other. In lobster, Johnson and Ache (1978) suggested such a receptor level antagonistic interaction between amino acids.

This study however confirmed that compounds which were found stimulants under laboratory conditions were also stimulatory under field conditions.

9. EFFECT OF STIMULANTS ON GROWTH PERFORMANCE

To ascertain the role of attractants and stimulants on growth performance feeding and growth trial was conducted using diets flavoured with the same. Hartati and Briggs (1993) also used feed intake, feed assimilation, growth, food conversion, and survival of shrimps to assess the growth promoting efficiency of feeding stimulant. Only such growth studies can determine whether stimulatory substance actually improve growth, food conversion, and survival. But Heinen (1980) stated that it may not be possible to ascribe such improvements to chemostimulants, since addition may also play some useful metabolic role. But, since we are considering most of feed intake, assimilation and other growth parameters together, it is very easy to identify such metabolic role.

Incorporation of various stimulant mixture in the semi-purified compound diet improved its palatability and growth performance (Table 53, Fig28, 19). Growth of the shrimp was fast for diets flavoured with chemostimulants compared to its control, from initial stage of rearing (Fig 28, 29). In the present study the improved growth rate followed an increased in feed intake, food conversion and feed assimilation efficiency indicating the positive role

of chemostimulants on feed utilization and growth. In most of the cases the growth performance of the shrimps in response to chemostimulants followed more or less similar trends on that observed during behavioural evaluation. Hartai, and Briggs (1993) in P.monodon observed similarities to the effect of attractant supplementation of feed on subsequent on growing performance with the behavioural responses of shrimps to feeding attractant.

9.1 FEED INTAKE AND ASSIMILATION

Incorporation of chemoattractants and/or stimulants in the feed increased feed intake and assimilation in P.incidus than the corresponding control diet in the case of both semipurified and control diets. Similar increase in feed intake and assimilation was also reported for other shrimps like P.japonicus (Deshimaru and Yone, 1978) P.monodon (Hartati and Briggs, 1993) M.rosenbergii (Harpaz et.al., 1987; Smith et.al., 1987; De Proenca 1990) and in P.pugio (Carr and Thompson, 1983) when the feeds were incorporated with selected feeding attractants or stimulants.

The feeding attractant and stimulants, stimulate the secretion of digestive enzymes (gastric juice) by hepatopancreas. The increased production of digestive enzymes change digestion rate and thus improved the appetite of the animals, which in turn increased feed intake. The enhanced digestion also improved the absorption of nutrients into the body and thus increased feed assimilation efficiency by minimising feed wastage through faeces.

9.2 GROWTH

The result indicated that attractant and or stimulants in the diet not only impound its palatability P.indicus but increased its growth and specific

growth rate also. Several workers reported improved growth in P.monodon when the diets were supplemented with different stimulants (Pascual, 1980; Murai et.al., 1981, 1985; SEAFDEC, 1990; Hartati and Briggs, 1993). These authors suggested that tissue extracts of prey organisms contains large quantities of taurin, and a mixture of amino acids, which were very attractive to P.monodon and increased growth and survival rates due to enhanced diet acceptability. SEAFDEC, (1990) reported that diet incorporated with a potential chemoattractants produced highest survival weight gain, and specific growth rate. Similar improved growth rate was also observed in M.rosenbergii when arginine was incorporated into the diet (Farman - Farmaian, 1979). Heinen (1980) stated that the increased growth rate may be due to the fact that the increased growth rate may be due to the fact that addition of proteins and other essential amino acids to a diet deficient in these nutrient might have a role in the metabolic process as well as acting as incitants. However many of the attractants used, such as taurin, Finnsim and betaine-glyine mixture was not able to promote such a response (Coloso and Crz, 1980).

The improved growth and specific growth rate formed in the present study is due to increased feed intake and assimilation produced by the attractant and/or stimulants.

9.3 FOOD CONVERSION

Analysis of the food conversion ratio of shrimp feed with the test diets revealed similar results to those with the growth rate. This measurement thus seemed to be a more sensitive indicator of shrimp feeding efficiency than assimilation efficiency. In P.monodon, SEAFDEC (1990) and Hartati and Briggs (1993) who obtained similar better FCR with diet incorporated with potential chemoattractants; than the corresponding unflavoured diet. But in

M.rosenbergii, Farman-Farmaian et.al. (1979) found that incorporation of arginine stimulated growth but did not affect food conversion, but lysine stimulated growth with an understiable increase in the food conversion ratio.

9.4 **SURVIVAL**

The survival of shrimps fed with diets containing potential demostimulants were significantly higher than that for unflavoured diets. This increased survival associated increased growth in due to the enhanced palatability of the diets. Such increase in survival rate was also reported by several workers when diets were supplemented with chemoattractants or stimulants in P.monodon (Pascual, 1980; Murai et.al., 1981; Hartati, and Briggs, 1993)

A comparison of data from behavioural and growth trials revealed similarities in the ranking of the attractiveness of the test diets. This suggests that behavioural studies may be able to predict to some extent subsequent on growing performance.

Summary

SUMMARY

1. To identify and evaluate the chemotactic property of various feeding stimuli for Penaeus indicus and Metapenaeus dobsoni a series of behavioral and feeding trials were conducted under laboratory and field conditions.

2. A series of behavioural descriptors like perception, orientation, displacement, arrival and ingestion were developed for the behavioural evaluation of chemoreception in shrimps.

3. Behavioural responses like flicking of antennule, walking or swimming, grasping and lifting movements of pereopods and movements of mouth appendages have been used as the behavioural indicators related to feeding in the study.

4. Shrimp activity recorder was used to quantify and record the activity of shrimps.

5. To classify chemical stimuli based on their feeding property on attractant and repellent; two chemotactic indices; Db and Rb were developed.

   * The Db value for attractants will be always above zero and the same for repellent below zero.
   * The Rb value will be always above '1' and below '1' respectively for attractants and repellents.
   * Db value zero or an Rb value '1' indicate neutral activity of a sample.

6. The time required to elicit various feeding behaviours decreased and the response of the shrimp increased with the concentration of the attractant, whereas in the case of repellent, the time lag to elicit feeding behaviour increased and the response decreased sharply.

7. Evaluation of the extracts of natural materials showed that they are attractive to P.indicus and M.dobsoni.

8. Tissue extracts at the same concentration differed significantly in their property to elicit feeding response in both the species. P.indicus showed maximum response to squid extract and M.dobsoni to shrimp extract, with small EC_{50} and ET_{50} values.

9. The major attractant substance present in the tissue extracts were free amino acids with, 50% and 78.2% of the total activity of the extracts in M.dobsoni and P.indicus respectively.

10. Protein fraction contribute 12.8% and 17.29% and lipid fraction 3% and 6.75% of the chemotactic property of extracts in P.indicus and M.dobsoni respectively.

11. L-isomers of amino acids were chemotactically more active than DL-forms and for eliciting the same amount of feeding response DL-forms require higher concentration than L-forms.

12. Amino acids like lysine, methionine, glycine, alanine and proline in P.indicus and lysine, methionine, alanine, phenylalanine and leucine in M.dobsoni produced highest feeding response.

13. The threshold concentration of L-amino acids ranged between $4x10^{-2}$M to $1x10^{-10}$M in P.indicus and 1.5 M to $1x10^{-10}$ in M.dobsoni. Whereas in the case of DL-amino acids, it ranged between $1x10^{-2}$ to $1x10^{-5}$ M for both species.

14. Amino acids like glycine, phenylalanine, lysine and histidine function as feeding attractant.

15. Glutamic acid, serine, isoleucine, arginine, leucine, aspartic acid, proline, lysine and methionine function as incitant.

16. Glycine, alanine, taurine, tryptophan, proline, lysine and isoleucine functioned as stimulant and cysteine, valine and betaine as arrestant.

17. Most of the amino acids produced only one of the above property, but some amino acids like glycine and lysine acts as attractant, incitant and stimulant in the same animal at different concentrations. At low concentration above threshold concentration they act as attractant and at still higher concentration as incitant. But at a still higher concentration they function as stimulant.

18. Salinity is found to have some influence on the chemoreceptive property of the shrimps with 15 to 25‰ salinity as the most ideal for feeding activity, producing higher feeding response and feed intake in both the species.

19. Behavioural responses of the shrimps to the feeding stimuli varied significantly with pH, being more responsive at pH 8.0 and least at pH 6.0 and 10.0.

20. At pH 6.0 and 10.0 perception, orientation and swimming activity became very weak, with a longer time lag to elicit the feeding response.

21. The sharp decline in the feeding response at the extreme pH levels were due to the destruction of sensory epithelium.

22. pH significantly influenced the feed intake by the shrimps with maximum at pH 8.0 and minimum at pH 6.0 and 10.0. At pH 6.0 and 10.0 the feed intake reduced by 50% than that at pH 8.0.

23. The chemoreceptiveness and feeding response increased with the degree of starvation, thereafter it declined considerably due to the physical weakness of the test animals.

24. Blocking experiments with the shrimp appendages indicated that the major chemoreceptor sites concerned with feeding seems to be on the antennule, on the distal portion of the pereopods and on the mouth appendages.

25. Both the inner and outer ramous of the antennules are chemosensory and are involved in distance chemorception which mediate arousal and search behaviour.

26. Two morphologically distinct setae types are present on the ramous, of which the thin walled aesthetasc is involved in distance chemoreception.

27. Propus and dactylus of the first three pairs of walking legs are chemosensory, involved mainly in contact demoreception. It is also involved in distance chemoreception. The walking leg chemoreceptors mediate mainly seizure and conveyance of food particles to the mouth.

28. Two morphologically distinct Setae types responsible for contact chemoreception and a branched thin walled setae involved in distance chemoreception are present on the propus and dactyl.

29. Behavioural and morphological studies indicated that mouth appendages are also chemosensory, involved in contact and distance chemoreception, and mediate ultimate acceptance or rejection.

30. Morphological observations of the chemoreceptor site indicated that maxilla I and II and maxilliped 1 and III are contact chemoreceptors, whereas maxilliped III acts mainly as distance chemoreceptors. Mandibular palp bear aesthetasc hairs similar in structure to that involved in distance chemoreception on the pereopods.

31. The agar matrix bioassay served as an efficient method to screen a wide variety of attractants and stimulants.

32. Tissue extracts produced more or less the similar pattern of ingestion activity, as that observed during the behavioural studies.

33. Free amino acid fraction form the major flavour component of the tissue extracts eliciting ingestion activity, producing 47.5 to 68.6% of the feeding activity of the whole extract in M.dobsoni and 45.5 to 57.2% in P.indicus.

34. Among the various amino acids neutral and basic amino acids produced maximum ingestion activity.

35. The other major flavour components of the tissue extracts after amino acids are nucleotides and soluble proteins.

36. Among the nucleotides, Inosine mono phosphate (IMP) produced maximum ingestion activity in both species.

37. Methoionine, lysine, proline,arginine and aspartic acid in P.indicus and aspartic acid, methionine, leucine, tryptophan and isoleucine, in M.dobsoni produced maximum ingestion activity.

38. L-isomers of amino acids produced higher ingestion activity than the corresponding DL-aminoacids.

39. Betaine, glycine, and inosine produced synergestic effect on feeding stimulation activity, when supplemented with other amino acids and their mixtures, due to additive activity of the amino acids on the receptor sites; than when they were supplemented individually.

40. Certain amino acid mixtures produced poor performance than their individual components indicating antagonism in feeding stimulation activity, due to competition among amino acids for common receptor sites neutralizing the activity of each other.

41. Synthetic crab extract produced maximum ingestion activity and feed intake in P.indicus followed by the squid and shrimp extract.

42. Flavouring the diets with an attractant significantly reduced the time lag to attract the shrimps to the diet and to initiate ingestion activity.

43. Supplementation of selected feeding stimulants in compounded diets improved its palatability, resulting in increased feed intake, feed assimilation efficiency, specific growth rate, food conversion and growth under laboratory conditions.

44. Incorporation of flavourant compounds in the feed also increased the palatability and feed consumption in grow out ponds compared to unflavoured control.

45. Standard compounded diet with mixed protein base, performed better in their palatability and growth performance than single meal based diets.

46. Cost consideration and optimum level of some amino acids suggested that appropriate concentration of chemoattractant/stimulants, will be about 1% or below.

47. The increased feed cost may be offset by improved growth performance and this is by making the food more palatable with chemical stimuli.

48. Results indicated that the entire sequence of feeding behaviur from appetetive behavioural patterns to the consumatory act of feeding can be released by external chemical agents alone.

49. Present results also indicated that behavioural studies may be able to predict subsequent on growing performance.

50. Both P.indicus and M.dobsoni differ qualitatively and quantitatively in their chemoreceptiveness, and in response to different chemical stimuli.

51. Post-larvae, juveniles and sub-adults of both species differeed in their chemoreceptiveness, with juveniles being chemotactically more responsive.

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CHEMORECEPTION STUDIES IN RELATION TO FEEDING RESPONSES IN THE MARINE SHRIMPS *PENAEUS INDICUS* H. MILNE EDWARDS AND *METAPENAEUS DOBSONI* MIERS.

ABSTRACT

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ABSTRACT

Chemoreception in relation to feeding and the various factors involved in the same in Penaeus indicus and Metapenaeus dobsoni were investigated under laboratory and field conditions by behavioural and ongrowing studies. The entire sequence of feeding behaviour in shrimps from appetitive behavioural pattern to the consumatory act of feeding can be released by chemical stimuli alone. The chemotactic indices, Db and Rb, developed were used to classify the feeding stimuli as attractant and repellent based on their chemotactic property.

Analysis of extracts of natural food materials for both species showed that they are attractive to P.indicus and M.dobsoni, whereas the squid ink acted as a feeding repellent for shrimps. The feeding response increased with increase in extract concentration and decreased with squid ink concentrations. The major attractant and stimulant substances present in the extracts were free amino acids with 78.2% of the extract activity in P.indicus and 50.28% in M.dobsoni, and nucleotides. Soluble proteins and peptides, lipids and carbohydrates also evoke feeding response but at lower level.

Among the various groups of aminoacid; neutral followed by basic amino acids and among nucleotides; Inosine Mono Phosphate (IMP) produced maximum feeding response. At the same concentration L-Amino acids were found more stimulatory than the corresponding DL-amino acid. The threshold concentration of L-amino acids ranged between 4×10^{-2}M and 1×10^{-10}M for P.indicus and 1.5 to 1×10^{-10}M for M.dobsoni, and in the case of DL-forms it is between 1×10^{-2}M and 1×10^{-5} M for both species. Amino acids like lysine, methionine, glycine, alanine and proline in P.indicus and lysine, methionine, alanine,

phenylalanine and leucine in M.dobsoni produced maximum feeding response and feed ingestion. Most of the amino acids have any one of the feeding activity, but those like glycine and lysine acted differently as attractants, incitant and stimulant at progressively increasing concentrations.

Environmental parameters like pH and salinity have pronounced influence on the chemoreception and feeding response in the shrimps, being chemotactically more active at pH between 7.0 and 9.0 and salinity between 15 and 25‰. The feed intake reduced by 50% at pH 6.0 and 10.0. The alertness towards feeding stimuli increased with the degree of starvation upto certain levels and thereafter decreased due to the physical weakness of the animal.

The agar matrix bioassay served as a cheap and efficient method to screen a wide variety of attractants and stimulants.

Flavouring the diets with potential natural and synthetic chemo attractants and stimulants reduced the time required to attract the shrimps to the feed and to initiate ingestion activity. It also improved the palatability and acceptability and subsequently improved food intake, growth, survival, food assimilation efficiency, specific growth rate and food conversion. This marked increase in the food intake and growth could be due to the increased digestive activity of the pancreatic secretion and the resultant increase in appetite.

The attractants and stimulants produced more or less the same pattern for ingestion activity as that elicited during behavioural trial. It also indicated that the ongrowing performance of various feeding stimuli could be predicted directly from the behavioural trial.

The chemoreceptors most concerned with feeding seemed to be on the antennules, on the pereopods and on the mouth parts. Antennule chemoreceptors were involved in distance chemoreception and to mediate arousal and search for potential food. The mouth and leg receptors functioned mainly as contact chemoreceptors; involved in the seizure and ingestion activity and to some extent they are also involved in distance chemoreception. Morphologically distinct chemosensory sensilla present on these appendages were the primary sites for chemoreception in these species.

Both P.indicus and M.dobsoni differed significantly in their chemotactic response to different stimuli. Among the various stages of animals studied juveniles were chemotactically more active than the post-larvae and juveniles.